2002

Effects of high-load eccentric exercise training on rat soleus muscle myofibrillar disruption following one-week of hindlimb suspension unloading and subsequent reloading

Rhonda Dianne Prisby
Louisiana State University and Agricultural and Mechanical College, rprisby@hotmail.com

EFFECTS OF HIGH-LOAD ECCENTRIC EXERCISE TRAINING ON RAT SOLEUS MUSCLE MYOFIBRILLAR DISRUPTION FOLLOWING ONE-WEEK OF HINDLIMB SUSPENSION UNLOADING AND SUBSEQUENT RELOADING

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College

in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Kinesiology

by

Rhonda Dianne Prisby
B.A., Hiram College, 1992
M.A., Kent State University, 1997
August 2002

ACKNOWLEDGEMENTS

My sincerest appreciation goes to my major professor Dr. Arnold Nelson for his support and guidance throughout my graduate studies as well as with this project. Many thanks to my other committee members, Drs. Robert Wood, Michael Keenan, Maren Hegsted and Changaram Venugopal.

Thanks to other graduate students for their emotional and educational support: Rafael Reyes, Mahmoud Alomari and William Edwards. The time we spent together in 44G will always be remembered and cherished.

I would like to extend a special thanks to the Human Ecology Department for allowing me access to the pDEXA and rat scale during my data collection and well as the staff at the Division of Laboratory Animal Medicine (DLAM) for their knowledge and assistance during data collection.

Lastly, I would like to acknowledge my family and friends for their unending support and encouragement in all of my endeavors. Further, I would like to dedicate this document to my parents: Richard Prisby and Thelma Williams Prisby.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Purpose: This investigation evaluated the effects of a high-load (50% body weight) eccentric exercise training protocol on reloading myofibrillar damage in soleus (SOL) and extensor digitorum longus (EDL) muscles in rats following 7 days (d) of hindlimb suspension unloading (HSU). **Methods:** 48 female Sprague-Dawley rats were randomly stratified to four experimental groups; exercise + hindlimb suspension unloading (ExHSU), hindlimb suspension unloading (HSU), exercise (Ex) and control (C). The ExHSU and Ex groups underwent a high-load eccentric exercise protocol for ~2.5 weeks. Following exercise training, the ExHSU and HSU groups underwent 7 d of hindlimb suspension unloading and a subsequent 16-19 h reloading period. ANOVA was used to determine significance between groups for the following variables: body weight (BW) across time, BW at sacrifice, Glucose-6-phosphate dehydrogenase (G-6-PDH) activity, fiber area, fiber area to body-weight ratio, % myofibrillar damage, SOL and EDL wet, dry and wet-weight to body-weight ratios, % interstitial area, adrenal weights and adrenal weight to body-weight ratios, tibia lengths and tibia bone mineral content. **Results:** ANOVA revealed no significant differences ($p > .10$) between the ExHSU and HSU groups for BW at sacrifice, fiber area, fiber area to body-weight ratio, SOL and EDL wet, dry and wet-weight to body-weight ratios, adrenal weights and adrenal weight to body-weight ratios and tibia lengths and bone mineral content. Yet a post analysis t-test revealed a significantly higher % of myofibrillar damage in the HSU vs. the ExHSU group. Further, G-6-PDH activity and % interstitial area approached

significance (p = 0.134 and p = 0.152, respectively). **Conclusions:** The high-load eccentric exercise training protocol prior to HSU attenuated the % of myofibrillar damage during reloading. Further, the % of interstitial area and G-6-PDH activity tended to be smaller in the ExHSU group vs. the HSU group. Therefore, eccentric exercise prior to HSU may elicit a repeated bout effect and attenuate the amount damage incurred by the muscle during reloading. Additionally, this investigation was the first to demonstrate increased G-6-PDH activity with reloading myofibrillar damage.

CHAPTER 1. INTRODUCTION

1.1. Rationale

Removal of mechanical loads (i.e., unloading) from the musculoskeletal system causes skeletal muscle atrophy [1]. Atrophy occurs in a variety of situations, which may include: prolonged bed rest [2, 3], aging and inactivity [4-6] and exposure to microgravity during space flight [7-9]. Musculoskeletal unloading causes alterations in muscle structure and function [10], which results in the loss of muscle strength [11-14]. Reductions in skeletal muscle size elicit structurally weaker contractile units (i.e., myofibrils) that are unable to support body weight and contractile activities similar to those experienced prior to either unloading or space flight. Hence, when weight-bearing activities are reestablished (i.e., when reloading occurs), skeletal muscle fibers are damaged [15].

Damage to skeletal muscle subsequent to periods of unloading has been observed in humans [16, 17], mice [2], rats [8, 18-31] and rabbits [32, 33]. Greater magnitudes of reloading damage have been directly associated with greater magnitudes of unloading atrophy [22, 25, 34]. In other words, higher incidences of reloaded skeletal muscle damage have been observed in muscle fibers with smaller diameters following unloading [25]. Further, skeletal muscle damage is a reloading phenomenon [28] and occurs only when the muscle assumes normal weight-bearing activities following the unloading period. Skeletal muscle myofibrillar damage has been observed as early as 2 min [32] into the reloading period as well as at 12-14 h [24], and between 12-48 h [28].

Unloading-induced atrophied skeletal muscles are most susceptible to damage caused by eccentric contractions [19, 21, 22, 35, 36] as opposed to concentric and isometric contractions. Researchers have noted numerous similarities between unloaded/reloaded skeletal muscle damage and damage related with delayed-onset muscle soreness (DOMS) [15, 37]. Delayed-onset muscle soreness (i.e., post-exercise muscle soreness) is described as the sensation of skeletal muscle discomfort and pain that occurs in a delayed fashion [38, 39]. The muscular discomfort and pain occurs following exercise that is novel to the individual (i.e., unaccustomed exercise) [38] and is usually most severe when the unaccustomed exercise includes eccentric muscle actions [37, 40]. The symptoms of DOMS (e.g., skeletal muscle stiffness, tenderness and aching) usually occur within the first 24-48 h, peaks between 24-72 h and dissipate within 5-7 d [41, 42]. The damage similarities between unloaded/reloaded skeletal muscle and skeletal muscle subjected to unaccustomed eccentric exercise include the following: increased number of damaged fibers [22, 29, 32, 43, 44], increased interstitial edema [8, 22, 45, 46], mononuclear cell infiltration [8, 28, 37, 46], localized (i.e., focal) disturbances in the cross-striated banding [19, 22-24, 28, 32, 37, 43-48] and abnormalities of sarcomeres and Z-lines [29, 32, 43, 44, 47-49]. Since the muscle morphology of unloaded/reloaded skeletal muscle damage and that observed in biopsied skeletal muscle following unaccustomed eccentric exercise share similar characteristics, perhaps the mechanisms of damage are similar.

Reports in the literature [50, 51] suggest that an initial bout of eccentric exercise alleviates skeletal muscle damage following a second bout. This phenomenon is known as the repeated bout effect (RBE). Exercise that causes skeletal muscle damage results in prompt adaptations within the muscle cell, resulting in the muscle becoming more resistant to the damaging effects of a second bout of the same exercise [50]. The prompt adaptations within the muscle cell may include: neural adaptations [52, 53], adaptations in connective tissue surrounding the muscle [50, 54, 55], adaptations in the cellular components of the muscle [51, 56], impairment of the calcium-mediated excitation-contraction coupling [57, 58], and a decline in the inflammatory response to injury [59] following the subsequent exercise bout. Since, unloaded/reloaded skeletal muscle damage is morphologically comparable to damage observed after unaccustomed eccentric exercise, the probability exists that initial bouts of eccentric exercise prior to skeletal muscle unloading may demonstrate the same prophylactic effects (i.e., repeated bout effect) as observed in previous investigations [50, 51]. Since this hypothesis had yet to be investigated in the literature, it was the aim of our laboratory to examine this question further. In pursuit of an answer, a preliminary and two pilot investigations were undertaken and the results utilized to formulate the hypothesis presented in this document.

1.2 Preliminary Investigation

In order to test the hypothesis that eccentric exercise prior to unloading would attenuate reloading skeletal muscle damage, our laboratory had to first 1)

evaluate the feasibility of experimentally inducing unloading atrophy, 2) determine the appropriate eccentric exercise protocol for a rat model, and 3) of additional interest, establish a criterion by which skeletal muscle damage could be quantified reliably within and between testers.

1.2.1 Inducing unloading atrophy: the hindlimb suspension unloading model

Several animal models (e.g., space flight, denervation, spinal cord transection, immobilization and hindlimb suspension unloading) are effective in limiting skeletal muscle activity and eliciting atrophy. The invasive natures of the denervation, spinal cord transection and immobilization models induces physiological alterations in conjunction to those changes induced by the experimental protocol, making it difficult to distinguish between the two. For example, spinal cord transection involves severing the spinal cord of the experimental animal [60, 61] and the immobilization model may limit the range of motion in a particular muscle by fixing (i.e., casting) the joint at a specific angle [3]. The invasive natures of these models and the infeasibility of conducting a space flight investigation made it impossible to utilize these techniques in our laboratory. Therefore, the non-invasive hindlimb suspension unloading (HSU) model was chosen as the experimental model by which to induce skeletal muscle atrophy in these series of pilot investigations.

The HSU model was specifically designed to study muscle fiber atrophy [62, 63] and was developed as a means to simulate a weightless environment [63]. Previous investigations, utilizing the HSU model and other decreased-use models,

have provided invaluable insight on the physiological adaptations that occur in the musculoskeletal system during unloading. Investigations have proven that muscles that have an antigravity function, cross a single joint, and contain a high percent of slow fibers are most susceptible to unloading skeletal muscle atrophy [63]. In accordance with above-mentioned factors, the vulnerability of particular skeletal muscles to unloading-induced atrophy can be predicted based upon the inherent characteristics (e.g., percent fiber type, function, etc.) of a given muscle. The hierarchy of unloading skeletal muscle atrophy is as follows: soleus (SOL) > gastrocnemius and plantaris > extensor digitorum longus (EDL) and tibialis anterior [64].

Due to the physiological, morphological and functional characteristics of the soleus (SOL) muscle, it is perhaps that most investigated skeletal muscle when studying unloading atrophy [19, 45]. The SOL contains a preponderance of slow-twitch muscle fibers, has an antigravity function and crosses a single joint [63]. Various magnitudes of SOL muscle atrophy have been reported in the literature. For example, SOL muscles in rats have atrophied 27% (6 d of HSU) [65], 29.3% (7 d of space flight) [19], 32% (22 d of space flight) [45], 45% (28 d of HSU) [25], and 62% (12.5 d of HSU) [28]. Similarly, SOL muscles in mice have atrophied 39% following 2 wk of limb casting [66].

In contrast to the SOL, the extensor digitorum longus (EDL) muscle is phasically active, does not have an antigravity function, and consists predominantly (~90%) of fast type fibers [63]. The EDL muscle is often

investigated in conjunction with the SOL to serve as a control [19, 45], because the effects of unloading on this muscle are less severe [45, 65]. For example, rat EDL muscles have atrophied 23.3% following 7 d [19] and 12% following 22 d of space flight [45]. Because of the morphological and functional characteristics and physiological responses of the SOL and EDL to periods of unloading, these muscles were selected for study in this investigation.

Since no previous investigation at Louisiana State University utilized the HSU model, the preliminary work included establishing an acceptable HSU methodology. In cooperation with the veterinary staff at the Division of Laboratory Animal Medicine (DLAM) and the Institutional Animal Care and Use Committee (IACUC) at Louisiana State University, an acceptable methodology of HSU in the rat model was established. This methodology was adapted from several different HSU models cited in the literature [62, 67, 68] and corresponded well with established criteria for an acceptable model of HSU [69]. Therefore, this model could be utilized in subsequent investigations in our laboratory without evoking significantly measurable stress in the suspended animal and without compromising expected experimental outcomes with stress-related physiological alterations. Illustrations of the HSU model utilized in our laboratory can be viewed in Appendix I.

1.2.2. Eccentric exercise protocol

The second aim of the preliminary investigation was the determination of an acceptable eccentric exercise training protocol in the rat model. The exercise

design was based upon previously published reports [70] that utilized ladder climbing to attenuate skeletal muscle atrophy in rats during HSU. In comparison to control animals, the exercising groups attenuated SOL atrophy during 7 d of HSU by 35% (7 d of ladder climbing at 85° grade, 50% body weight resistance, and 4 sets of 10 repetitions), 17% (7 d of ladder climbing at 85° grade, 75% body weight resistance, and 2 sets of 10 repetitions) and 24% (7 d of ladder climbing at 85° grade, 75% body weight resistance, and 4 sets of 8 repetitions) [70]. Since reloaded skeletal muscle damage is similar to damage observed following unaccustomed eccentric exercise [35, 37], an eccentrically biased activity (downhill walking) was selected for the preliminary investigation as opposed to ladder climbing (primarily a concentric activity). Further, the SOL undergoes a preponderance of eccentric contractions during eccentrically biased activities (downhill exercise) [37] and therefore, would undergo exercise training with this type of protocol.

The following eccentric exercise protocol was chosen for the preliminary investigation: 7 d of downhill walking at 85° grade, 75% body weight resistance, and 2 sets of 10 repetitions. However, modifications to the exercise protocol were made during the preliminary investigation. The modifications included altering the maximal angle at which the animals would descend the exercise apparatus and the maximal resistance (i.e., percent body weight resistance) the animals could safely carry down the grid. Also, the exercise training duration was adjusted from 1 wk (original design) to ~2.5 wk to allow for an exercise training familiarization

period. Therefore, the exercise protocol was conducted as follows: ~2.5 wk of downhill walking at 45° grade, 50% body weight resistance, and 2 sets of 10 repetitions. An example of the exercise apparatus can be viewed in Appendix I.

1.2.3 Quantifying muscle damage

The third objective of the preliminary investigation was to establish a reliable criterion by which skeletal muscle damage could be quantified within and between testers. Numerous investigations in the literature have utilized light and/or electron microscopic techniques to determine the extent of skeletal muscle damage [20, 24, 56]. However, interpretation of induced damage is subjective to observer and sampling bias and is difficult to objectively quantify. Muscle damage from electron microscopy is often illustratively reported in the literature [20, 23, 56] however, to our knowledge there were no established criteria by which electron microscopy can be used to quantify muscle damage. Based upon such reports and laboratory experience, a criterion for assessing muscle damage and the magnitude of damage was developed. This criterion was developed so that individuals unfamiliar with damage characteristics could analyze micrographs reliably with individuals more accustomed with the literature. Therefore, the objectives were to 1) establish a set of criteria by which muscle damage could be quantified using electron microscopy and 2) determine whether such criteria would be reliable within and between testers. Testers assessed damaged (D) vs. undamaged (U) sarcomeres and assessed the magnitude of damage on 11 electron micrographs. Sarcomeres were considered damaged if Z-line(s) were

abnormal [wavy, fragmented, missing or streaming] and if abnormalities were observed within the sarcomere [tear(s), infiltration, disruption of striated pattern, and/or overstretch in size]. The magnitude of damage was quantified by assessing the overall appearance of the sarcomere according to the given criteria and reports of ultrastructural skeletal muscle damage. The entire criteria and protocol can be viewed in Appendix J. Mixed model ANOVAs were used to determine intraclass correlation coefficients within and between testers. The results are presented in Table 1.2.3.

Table 1.2.3. Reliability within and between testers.

TESTERS	TRIALS (#)	D VS U	MAGNITUDE
1	3	r = .96	r = .90
2	3	r = .98	r = .91
3	3	r = .98	r = .95
Between	3	r = .85	r = .85

D = damaged, U = undamaged, magnitude = magnitude of damage.

The electron micrographs of the SOL muscle in the preliminary investigation displayed similar damage characteristic of those reported previously in the literature [20, 23, 24, 29, 56] and can be viewed in Appendix I (Figures I.7-I.9). The results indicate that the criteria established in this investigation for assessing skeletal muscle damage is reliable both within and between testers. More specifically, the intraclass correlation coefficient (ICC) ranges within trials for the testers were: r = .96 -.98 (D vs. U) and r =.90 - .95 (magnitude of damage). Likewise, the ICCs between testers were r = .85 (D vs. U) and r = .85 (magnitude of damage). Therefore, this method may be useful when an investigator is

attempting to obtain an overview of the magnitude of damage contained within a muscle.

In summary, the preliminary investigation demonstrated that an acceptable HSU model and eccentric exercise protocol in the rat model could be conducted successfully in our laboratory. Further, a reliable criterion for assessing the magnitude of skeletal muscle damage could be utilized for statistical comparison between groups.

1.3 Pilot investigation #1

The preliminary investigation assessed the 1) feasibility of experimentally inducing unloading atrophy by means of the HSU model, 2) determined an appropriate eccentric exercise protocol by which to carry out the proposed question, and 3) established a reliable criterion by which skeletal muscle damage could be quantified reliably and utilized to make statistical comparisons between groups. Therefore based upon the findings of the preliminary investigation, the first pilot study was conducted to 1) assess the effectiveness of the exercise protocol in attenuating reloading muscle (i.e., myofibrillar) damage, 2) utilize the established criterion to assess the magnitude of damage between those groups subjected to HSU, and 3) a new objective was to enzymatically quantify muscle damage between groups.

Enzymatic quantification of skeletal muscle damage has been used extensively in the literature [51, 71, 72] #57]. The release of several muscle-related enzymes (e.g. creatine kinase, lactate dehydrogenase, and glucose-6-

phosphate dehydrogenase) after damage or injury has been quantified through blood serum [71, 72]. Increased activity of glucose-6-phosphate dehydrogenase (G-6-PDH) has been associated with skeletal muscle inflammation, injury and repair. G-6-PDH activity has been specifically located in regions of nuclear accumulation and therefore the elevated G-6-PDH activity in damaged muscle was linked to the proliferation of cells involved in degenerative-regenerative processes [71].

Downhill running in rats induced increased activity of G-6-PDH [71]. Similarly, G-6-PDH activity increased in rat muscle interstitium following 3.5-4 h of walking on a motorized treadmill [73]. Hindlimb suspension unloading (HSU) induces muscle damage subsequent to periods of reloading, if the unloading period induced muscle atrophy. While this enzyme has been measured following exercise, to our knowledge this enzyme has not been measured in muscles subsequent to reloading damage. Therefore, the third objective of this pilot investigation sought to quantify differences in the measurement of G-6-PDH activity in rat SOL muscle following 7 d of HSU and a 16-19 h period of reloading. Previous investigations have visually assessed skeletal muscle damage during similar sacrifice times [24, 29, 35]. Therefore, skeletal muscle damage would be measurable at 16-19 h following the initial injury. Six female Sprague-Dawley rats were randomly stratified by weight into four groups: exercise + hindlimb suspension unloading (ExHSU; n=2), hindlimb suspension unloading: (HSU; n=1), exercise (Ex; n=1), and a control group (C; n=2). The variables measured in this

investigation were: SOL wet weight, SOL fiber circumference, the magnitude of damage measured between the ExHSU and HSU groups, G-6-PDH activity, body weight and adrenal gland weight. Adrenal gland weight is an indirect measurement of stress during the HSU treatment [70, 74-76]; therefore, a lack of statistical significance between groups indicates minimal stress during HSU. Table 1.3.1 depicts the experimental protocol utilized in this pilot investigation. Alpha level was set at $p < 0.05$. Based upon the previous literature:

- It was expected that SOL muscle wet weight would be lower in the suspended groups (ExHSU and HSU) when compared to the Ex and control groups.
- It was expected that the fiber circumference would be smaller in the suspended groups (ExHSU and HSU) vs. the Ex and control groups.
- It was expected that the HSU group would have greater G-6-PDH activity when compared to the ExHSU group. Further, both suspension groups (ExHSU and HSU) would have greater G-6-PDH activity than the Ex and control groups.
- It was expected that the magnitude of damage would be greater in the HSU group vs. the ExHSU group.
- It was expected that body weight at sacrifice would be lower in the ExHSU and HSU groups compared to the Ex and control groups.
- It was expected that adrenal gland weight would not differ between groups.

Table 1.3.1. Experimental protocol

2-3 day familiarization period: exercise with no added weight (ExHSU and Ex).
5 days of exercise with added resistance: Weight increased daily up to 50% BW (ExHSU and Ex).
5 days of exercise at 50% BW (ExHSU and Ex).
3 days of rest before hindlimb suspension unloading (All groups).
7 days HSU + 16-19 h reloading (ExHSU and HSU).

ExHSU; exercise + hindlimb suspension unloading, HSU; hindlimb suspension unloading, Ex; exercise, BW; body weight,

The low statistical power of the groups (ExHSU, n=2; HSU, n=1; Ex, n=1; C, n=2), precludes conclusive interpretation of the data. However, with the exception of G-6-PDH activity and body weight at sacrifice, the magnitude of damage (Figure 1.3.1) and SOL fiber circumferences (Figure 1.3.2) demonstrated the expected findings. In other words, the magnitude of damage was higher (p = 0.010) in the HSU vs. the ExHSU group (1675.0 $\pm$ 97.3 vs. 1273 $\pm$ 193.4, respectively). Further, SOL fiber circumferences were smaller in the HSU vs. the Ex group and smaller in the ExHSU vs. the Ex and control group (p = 0.000). SOL wet weight did not differ (p = .283) between the HSU group when compared to the other groups (Figure 1.3.3) and G-6-PDH activity approached significance (p = .117) between groups (Figure 1.3.4). Surprisingly, G-6-PDH activity was higher in the ExHSU vs. HSU group (5.96 $\mu mole \cdot g^{-1} \cdot min^{-1}$ vs. 2.17 $\mu mole \cdot g^{-1} \cdot min^{-1}$). Further, body weight at sacrifice demonstrated higher body weights in the ExHSU and HSU groups as opposed to the control group (Figure 1.3.5). However, the data indicates that the

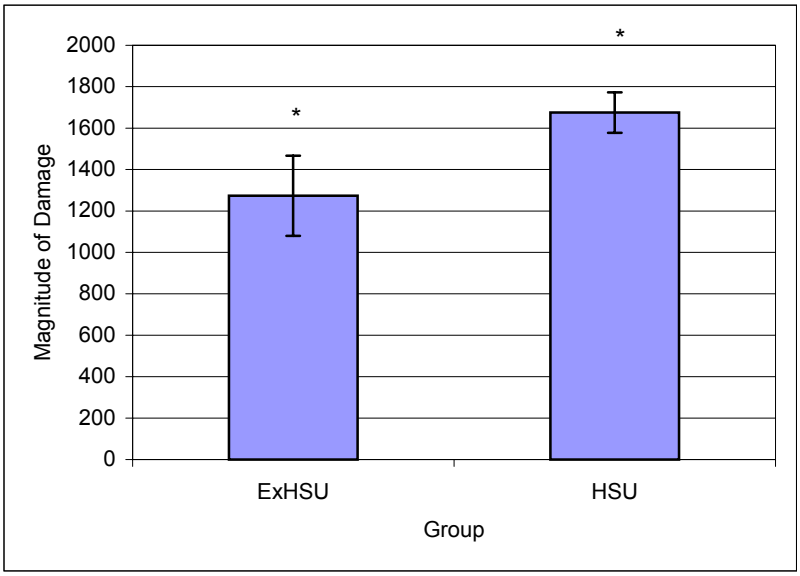


Figure 1.3.1 Magnitude of damage.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading.
*Denotes significant difference (p = 0.01).

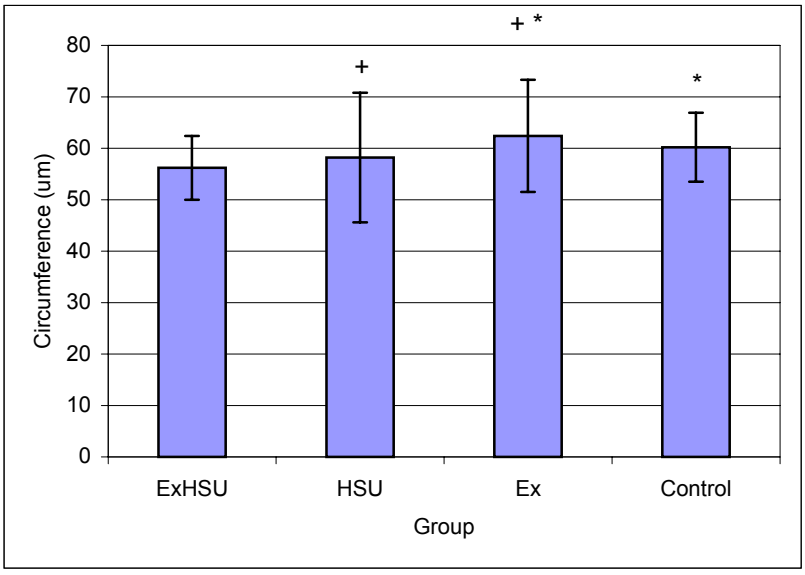


Figure 1.3.2 Soleus fiber circumference.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes significant (p<0.05) difference from ExHSU group.
$^{+}$Denotes significant (p<0.05) difference between the two groups.

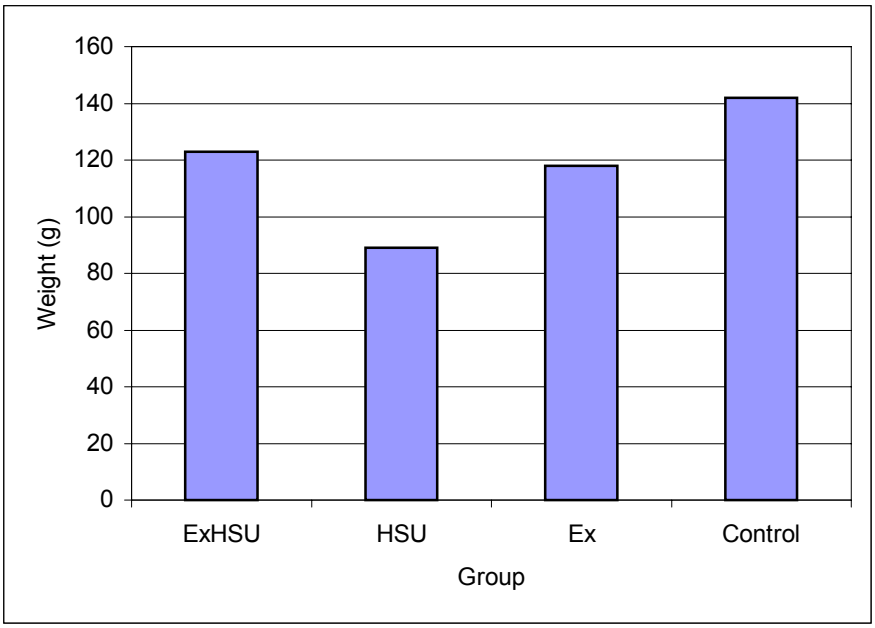


Figure 1.3.3 Soleus wet weight.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex,

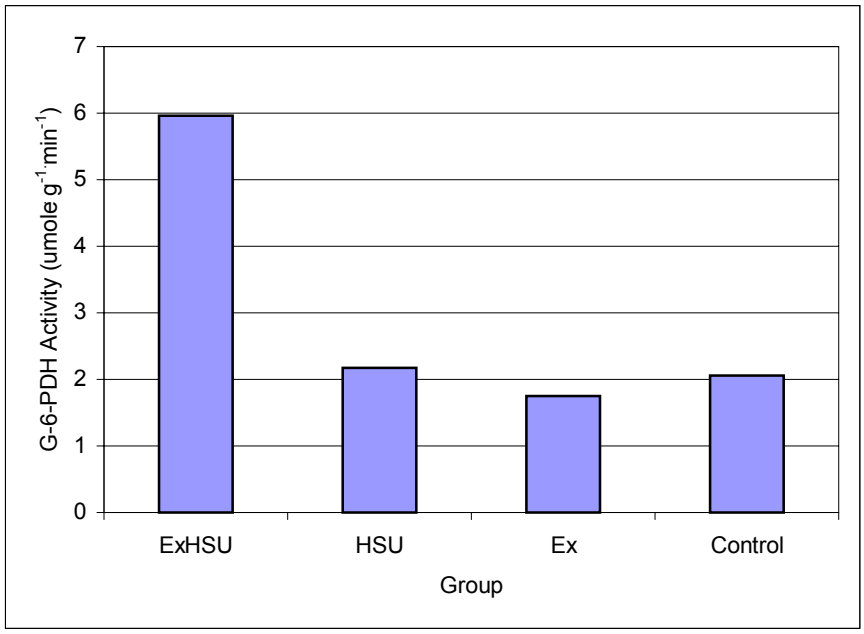


Figure 1.3.4. Glucose-6-phosphate dehydrogenase activity.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.

significance probably existed between the Ex group compared to all other groups. As anticipated, adrenal gland weight was not significantly different between groups. Therefore, the lack of a statistical difference indicates that stress to the suspended animals was minimal. Means, standard deviations and *p* values (when available), can be viewed in Table 1.3.2.

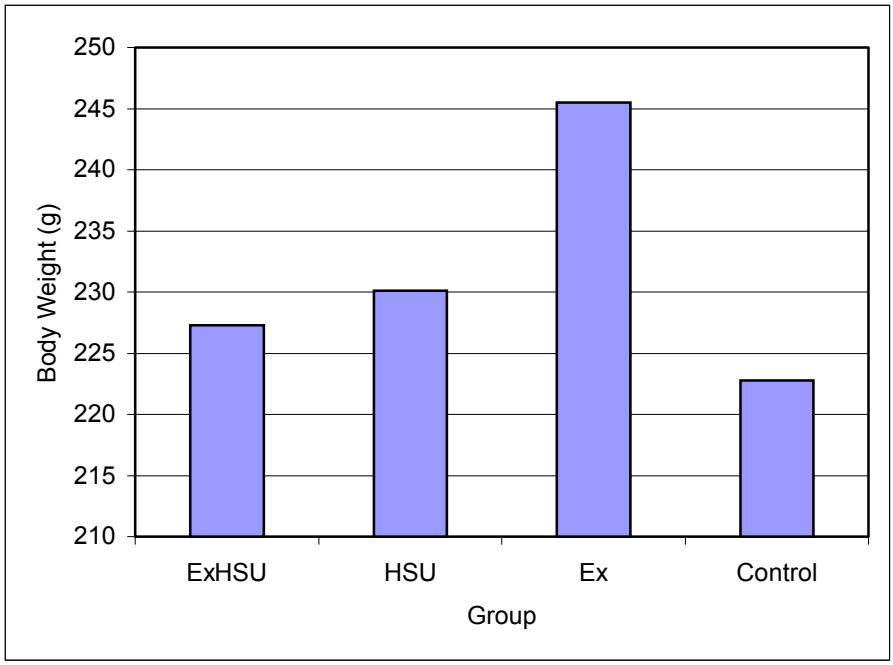


Figure 1.3.5. Body weight at sacrifice.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.

Figure 1.3.6 depicts the changes in body weight across time for each experimental group. However, the lack of a main effect (time, $p = .998$) and a statistical interaction (group x time, $p = .895$) should be noted. Separation of the data by experimental group allows for the presentation of a common physiological response often reported during the first 1-2 d of HSU. The rapid decline in body

weight in the suspended groups (ExHSU and HSU) is comparable to other reports of similar declines in body weight at the onset of HSU or space flight [9, 19, 75, 77-81]. The initial decline in body weight is transient, subsides within 3 d and has been attributed to marked diuresis [82, 83].

Table 1.3.2. Magnitude of damage, fiber circumference, enzyme activity, body and adrenal weights.

VARIABLE	GROUP				*p* =
	ExHSU	HSU	Ex	Control	
Magnitude of damage	1273.3±193.4‡	1675.0±97.3‡			0.010
SOL fiber circumference (μm)	56.2 ± 6.2	58.2 ± 12.6+	62.4±10.9*+	60.5±6.7*	0.000
SOL wet weight (mg)	122.6	89.0	117.6	141.6	0.283
G-6-PDH activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)	5.96	2.17	1.75	2.06	0.117
Body weight (g) at sacrifice	227.3	230.1	245.5	222.8	0.000
Adrenal gland weight (g)	.1083	.1347	.0803	.0425	0.352

Values represent mean ± standard deviations. Standard deviations reported if data available.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise; SOL, soleus; G-6-PDH, glucose-6-phosphate dehydrogenase.
The magnitude of damage only calculated between the ExHSU and HSU groups.
‡ Denotes significant difference between the ExHSU and HSU groups.
+Denotes significant difference between the HSU and Ex groups.
*Denotes significant difference from ExHSU group.

Summary of pilot investigation #1.

The results of this pilot investigation indicate that skeletal muscle damage during reloading may be attenuated with eccentric exercise prior to HSU. In other words, the magnitude of damage was lower in the ExHSU vs. HSU groups (*p* = 0.010). Yet G-6-PDH activity, an enzymatic quantification of muscle damage,

appeared to contradict this finding, as no statistical difference was observed. G-6-PDH activity approached significance ($p = 0.117$), however more activity was noted in the ExHSU group vs. all other groups. Further, G-6-PDH activity was similar between the HSU, Ex and control groups (2.17 $\mu mole \cdot g^{-1} \cdot min^{-1}$, 1.75 $\mu mole \cdot g^{-1} \cdot min^{-1}$, 2.06 $\mu mole \cdot g^{-1} \cdot min^{-1}$, respectively) and contradicts the expected outcome. However, technical inexperience in the measurement of this variable may have contributed to these unanticipated results.

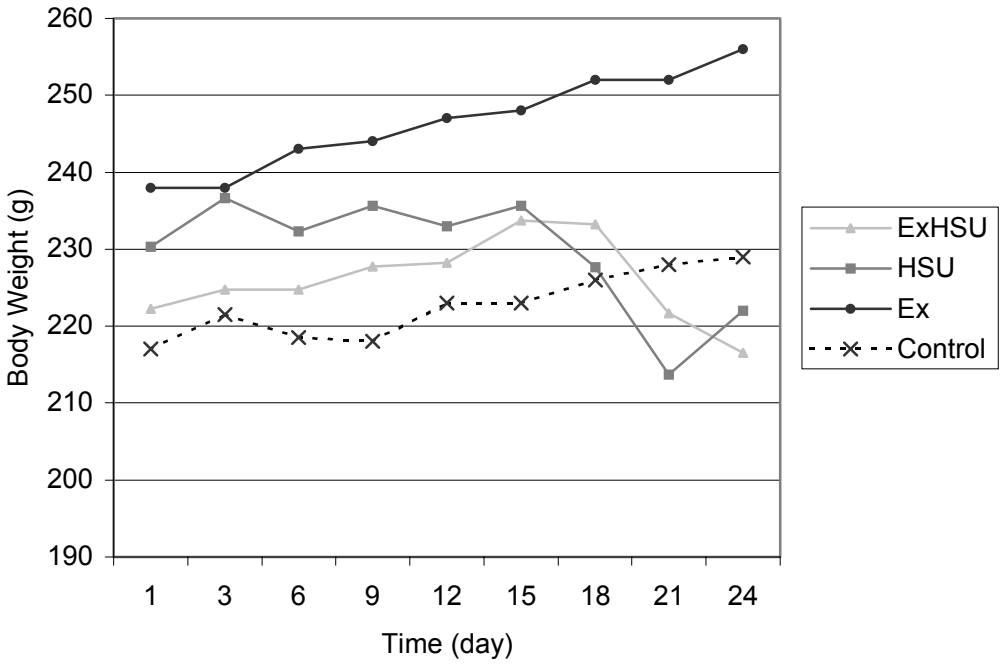


Figure 1.3.6. Body weight comparisons between groups.
ExHSU, exercise + hindlimb suspension unloading;
HSU, hindlimb suspension unloading; Ex, exercise.
Note: no significant interaction (group x time, $p = .895$).
The suspension period begun at day 17.

1.4. Pilot Investigation #2.

Results from pilot investigation #1 indicated that eccentric exercise prior to HSU might attenuate reloading damage (i.e., the magnitude of damage was significantly higher in the HSU group vs. the ExHSU group). However, the enzymatic data (G-6-PDH activity) contradicted this finding and demonstrated no difference between G-6-PDH activity between the HSU, Ex and control groups. Further, G-6-PDH activity was highest in the ExHSU group. Therefore, the purpose of the 2nd pilot investigation was to determine whether G-6-PDH activity was a reliable enzymatic measure of skeletal muscle damage. This investigation also included the examination of the extensor digitorum longus (EDL) muscle. Since less atrophy is often reported in the EDL compared to the SOL muscle [19, 22, 65], the EDL muscle serves as a control when examining the validity of the HSU model. Further, as opposed to the electron microscopy utilized in the previous investigation, this investigation utilized light microscopy to examine the percent of myofibrillar (i.e., muscle) damage between the HSU and control groups. Due to the small number of micrographs (i.e., ExHSU; n = 5, and HSU; n=5) analyzed for each muscle in the previous pilot investigation, a true representation of the overall amount of damage within the muscle might not be accurate utilizing this method. Therefore, the quantification of muscle damage with light microscopy would provide a larger representative sample of myofibrillar damage in the SOL muscle (i.e., approximately ¼ of the entire SOL muscle was analyzed).

This pilot investigation consisted of a hindlimb suspension unloading group (HSU; n = 4) and a control (C; n = 6) group. The HSU group was suspended for 7 d followed by a 16-19 h reloading period prior to sacrifice. The variables measured in this investigations consisted of the following: percent myofibrillar damage in the SOL muscle, G-6-PDH activity in the SOL and EDL muscles, SOL fiber area, SOL and EDL wet and dry weights, SOL and EDL wet-weight to body weight ratios, body weight and adrenal gland weight. The measurement of muscle wet-weight to body-weight ratio allows for the examination of muscle growth (or atrophy) relative to the overall growth of the animal (i.e., the gain in muscle weight relative to the gain in body weight). Smaller muscle wet-weight to body-weight ratios are indicative of muscle atrophy. Alpha level was set at $p \leq 0.10$. Based upon previous literature and the first pilot investigation, it was expected that:

- The percent of SOL myofibrillar damage would be higher in the HSU vs. the control group.
- SOL fiber area would be smaller in the HSU vs. the control group.
- SOL G-6-PDH activity would be higher in the HSU vs. the control group.
- EDL G-6-PDH activity would not differ between the HSU and control groups.
- Body weight would be smaller in the HSU vs. the control group.
- SOL muscle weights and SOL muscle wet-weight to body-weight ratio would be smaller in the HSU vs. the control group.

- EDL muscle weights and EDL muscle wet-weight to body-weight ratio would not differ between the HSU and control group.
- Adrenal weights would not differ between the HSU and control groups.

Data from this pilot investigation are presented in Tables 1.4.1 through 1.4.8 and graphs 1.4.1 through 1.4.5. The percent myofibrillar damage, SOL and EDL G-6-PDH activity, SOL fiber area, and adrenal gland weights correspond with the expected findings. The percent myofibrillar damage calculated for the HSU and control groups is presented in Table 1.4.1. The total muscle area measured did not differ (p = .162) between the groups. However, percent myofibrillar damage was significantly smaller (p = .077) in the control group compared to the HSU group. Figure 1.4.1 depicts this difference. Due to the large standard deviations presented with the one-way ANOVA, a Mann-Whitney U analysis was performed on the data. The results of this analysis are presented in Table 1.4.2. These results also demonstrated a significant (p = .039) difference between the HSU and control groups (Figure 1.4.2).

Table 1.4.1. Soleus longitudinal fiber damage.

VARIABLE	GROUP		p =
	HSU	Control	
Total Muscle Area Measured (mm^2)	158235.7 $\pm$ 17630.1	174726.6 $\pm$ 13702.8	.162
Percent Damage (%)	5.93 $\pm$ 6.55*	0.59 $\pm$ 1.21*	.077

Values represent mean $\pm$ standard deviation.
*Denotes a significant difference between the groups.
HSU, hindlimb suspension unloading.

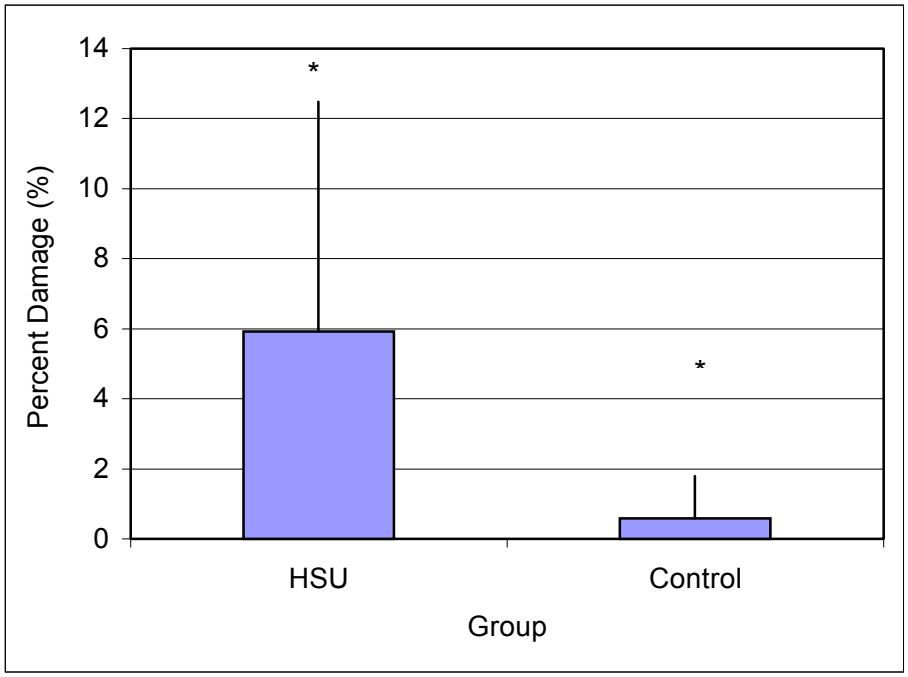


Figure 1.4.1. Percent myofibrillar damage of the soleus.
HSU, hindlimb suspension.
*Denotes significant difference between groups at $p = 0.077$.

Table 1.4.2. Mann-Whitney U ANOVA on ranks.

VARIABLE	GROUP		p =
	HSU	Control	
Percent Damage (%)	7.67*	3.67*	.039

Median value represented.
HSU, hindlimb suspension unloading.
*Denotes significant difference between groups at $p = 0.039$.

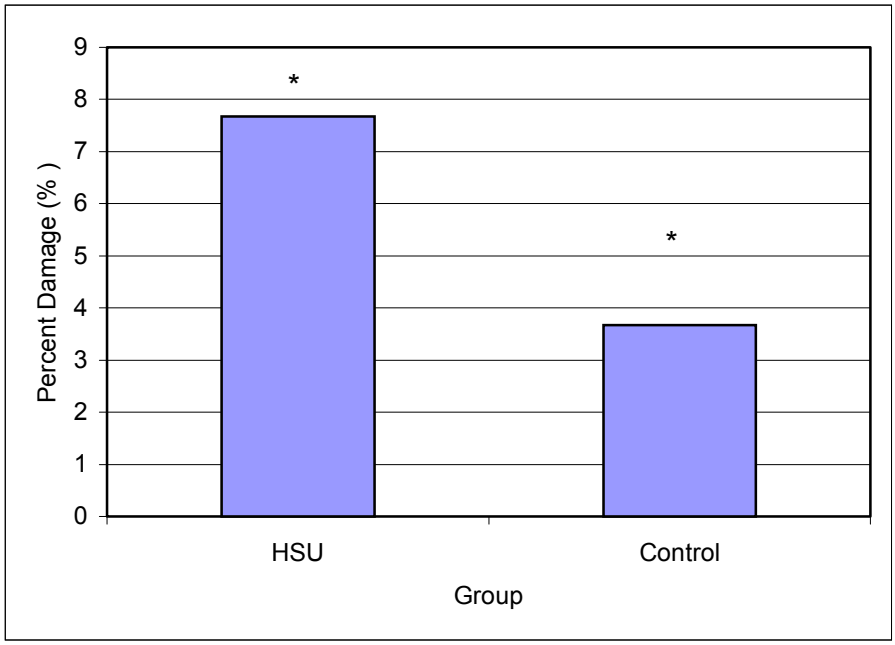


Figure 1.4.2. Percent myofibrillar damage determined by the Mann-Whitney U ANOVA on ranks.
HSU, hindlimb suspension.
*Denotes significant difference between groups $p = 0.039$.

As anticipated, EDL G-6-PDH activity did not differ ($p = 0.530$) between the HSU and control groups (Table 1.4.3). Yet, SOL G-6-PDH activity (Figure 1.4.3) was higher in the HSU group vs. the control group (3.56 $\pm$ 1.15 μmole$\cdot$g$^{-1}\cdot$min^{-1} vs. 2.10 $\pm$ 0.814 μmole$\cdot$g$^{-1}\cdot$min^{-1}, respectively). Likewise, SOL fiber area was smaller ($p = 0.095$) in the HSU vs. the control group (Figure 1.4.4). Mean fiber areas are presented in Table 1.4.4.

Table 1.4.3. Glucose-6-phosphate dehydrogenase activity (μmole$\cdot$g^{-1}min^{-1}).

VARIABLE			*P* =
	HSU	Control	
SOL	3.56 $\pm$ 1.15*	2.10 $\pm$ 0.814*	0.059
EDL	0.874 $\pm$ 0.380	1.23 $\pm$ 0.862	0.530

Values represent means $\pm$ standard deviation.
SOL, soleus; EDL, extensor digitorum longus; HSU, hindlimb suspension unloading.
*Denotes significant difference between groups.

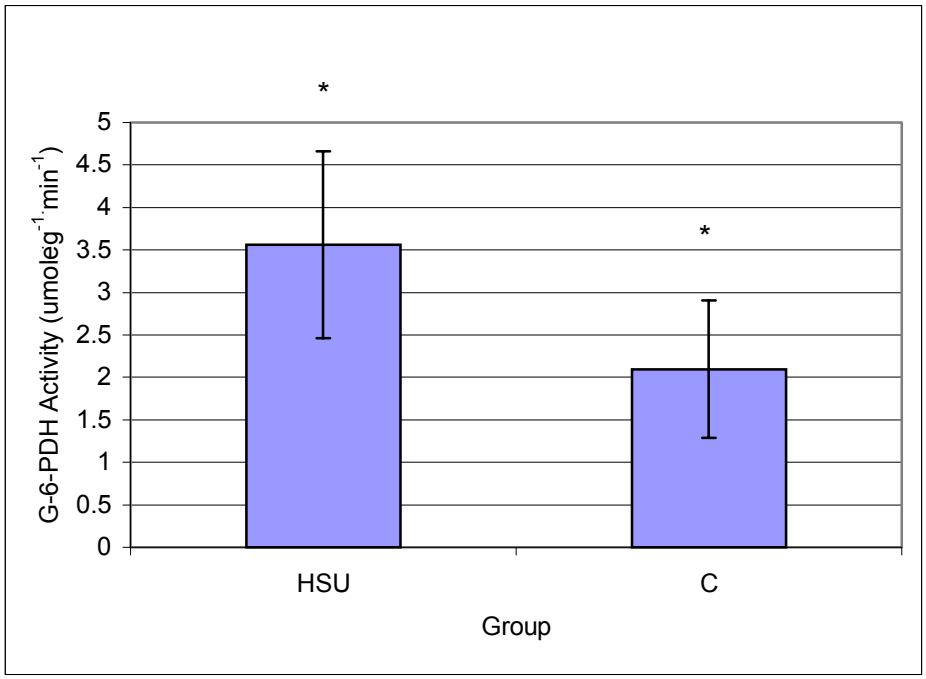


Figure 1.4.3. Soleus glucose-6-phosphate dehydrogenase activity.
HSU, hindlimb suspension unloading; C, control.
*Denotes significant difference between HSU and control groups.

Table 1.4.4. Mean soleus fiber area.

VARIABLE	GROUP		*P* =
	HSU	Control	
Fiber Area (um^2)	1576.4 ± 353.8	2988.5 ± 1167.5	0.095

Values represent mean ± standard deviation.
HSU, hindlimb suspension unloading.

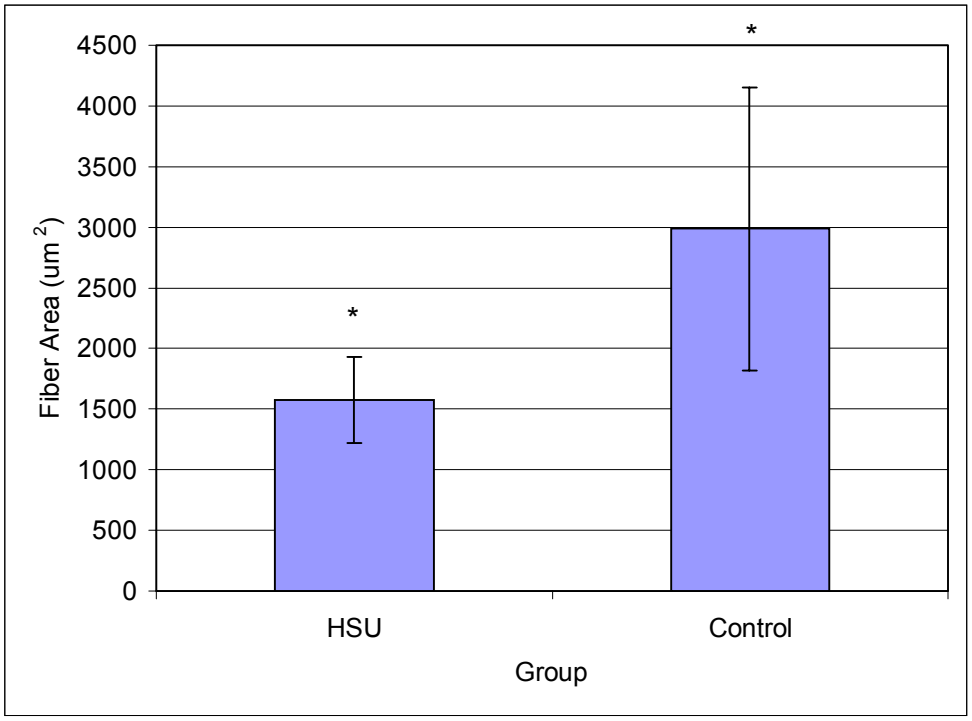


Figure 1.4.4. Mean fiber area.
HSU, hindlimb suspension unloading.
*Denotes significant (p = .095) difference between groups.

Body weight (BW) did not differ between groups at the start of the experimentation (p = 0.213), one day prior to suspension (p = 0.367), nor at sacrifice (p = 0.874) (Table 1.4.5). Further, body weight in the HSU group did not differ significantly from the control group across time (group x time, p = 0.831). In other words, the HSU group did not gain or lose more weight during the course of the experimentation than the control group. Figure 1.4.5 depicts a main effect in body weight over time, indicating that the HSU rats gained body weight similar to control rats.

Table 1.4.5. Mean body weight.

VARIABLE			*p* =
	HSU	Control	
Initial BW (g)	174.3 ± 17.8	157.2 ± 20.6	.213
BW (g) prior to HSU	215.8 ± 19.2	204.5 ± 17.3	.367
BW at Sacrifice (g)	210.00 + 8.66	212.50 + 24.84	.874

Values represent means ± standard deviations.
BW, body weight; HSU, hindlimb suspension unloading.

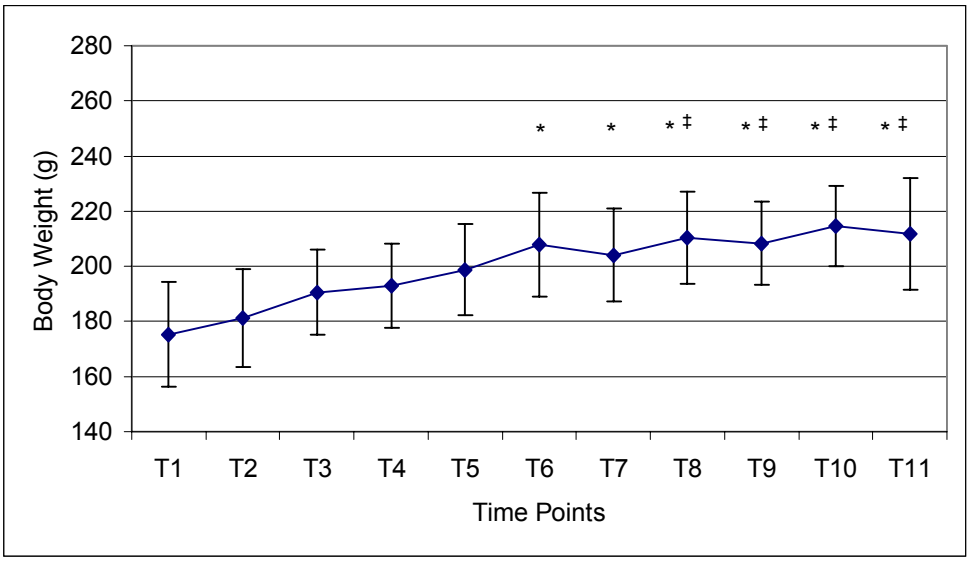


Figure 1.4.5. Body weight across time.
*Denotes significant difference ($p < 0.10$) from time point 1.
‡Denotes significant difference ($p < 0.10$) from time point 2.

Similar to body weight at sacrifice, soleus muscle weights (wet and dry) were not different between the HSU and control groups. Further, soleus wet-weight to body-weight ratio was not significantly different between groups. As anticipated, EDL wet and dry weights and wet-weight to body weight ratio was not significantly different between groups. Mean muscle weights and wet-weight to body-weight ratios can be viewed in Table 1.4.6. Further, adrenal gland weights

did not significantly differ between the HSU and control group, as depicted in Table 1.4.7.

Table 1.4.6. Mean muscle weights.

VARIABLE	HSU	Control	*P* =
SOL Wet Weights (g)	.0988 ± .0173	.0971 ± .0170	0.891
SOL Dry Weights (g)	.0655 ± .0225	.0674 ± .0137	0.879
SOL wet-weight to body-weight ratio ($g \cdot kg^{-1}$ BW)	.4694 ± .0690	.4556 ± .0460	0.725
EDL Wet Weights (g)	.0844 ± .0077	.0827 ± .0739	0.846
EDL Dry Weights (g)	.0522 ± .0188	.0535 ± .0194	0.917
EDL wet-weight to body-weight ratio ($g \cdot kg^{-1}$ BW)	.4021 ± .0326	.3875 ± .0251	0.476

Values represent mean ± standard deviation
SOL, soleus; BW, body weight; HSU, hindlimb suspension unloading; EDL, extensor digitorum longus.

Table 1.4.7 Mean adrenal weight.

VARIABLE	GROUP	
	HSU	Control
Adrenal Weights (mg)	82.9 ± 15.0	70.3 ± 18.0

Values represents means ± standard deviation.
HSU, hindlimb suspension unloading.

In summary, the results of this pilot investigation demonstrated that G-6-PDH activity is higher in the HSU group vs. the control group. Further, percent myofibrillar damage was higher in the HSU group compared to the control group. Therefore, G-6-PDH activity can be utilized to detect enzymatic changes in damaged muscle tissue following HSU and reloading. Likewise, light microscopy is an effective tool for the analysis of myofibrillar damage.

1.5. Summary of the preliminary and pilot studies

Data from the preliminary and pilot investigations indicate that eccentric exercise prior to HSU may attenuate reloading myofibrillar damage. This theory is

supported by the higher magnitude of damage observed in the HSU vs. the ExHSU group. Similarly, reloading following HSU corresponded to higher percents of SOL myofibrillar damage and higher SOL G-6-PDH activity in the HSU group vs. the control group. As anticipated, SOL fiber areas and circumferences were significantly smaller following suspension. Further, body weight significantly increased across time and there was no difference in adrenal gland weight, which indicates that stress during HSU was minimal. However, limitations of these investigations include the small sample sizes and power of the experimental groups. Thus, to accurately determine whether eccentric exercise prior to HSU will attenuate reloading myofibrillar damage, another investigation that increases the statistical power must be undertaken. Therefore, based upon the preliminary and pilot investigations and previous reports in the literature, it was hypothesized that:

1. The high-load eccentric exercise training protocol prior to hindlimb suspension unloading (ExHSU) would cause a differential response to reloading when compared to unloading without prior exercise (HSU).The differential responses would occur in:
    a. A higher percent myofibrillar damage measured in the HSU group vs. the ExHSU group,
    b. A higher percent interstitial area in the HSU group vs. the ExHSU group, and
    c. Higher G-6-PDH activity in the HSU group vs. the ExHSU group.

2. The EDL muscle would not demonstrate a differential response between groups with muscular reloading. In other words, no significant change in:
    a. G-6-PDH activity between groups (ExHSU, HSU, Ex and control).

CHAPTER 2. REVIEW OF LITERATURE

2.1 Skeletal Muscle Unloading and Reloading

Various organ systems are affected by periods of unloading. Unloading is defined as the removal of mechanical loads from the musculoskeletal system that causes skeletal muscle atrophy [1]. In contrast, reloading is defined as the reestablishment of mechanical loads on the musculoskeletal system following a period of unloading [81]. Atrophy elicited by unloading occurs in a variety of situations, which include: prolonged bed rest [2, 3], aging and inactivity [4-6], and exposure to microgravity during space flight [7-9]. The unloading of skeletal muscles cause alterations in structure and function [10], resulting in loss of strength [11-14] and in severe cases the demineralization of bones [78]. In fact, significant muscle atrophy is present in humans in as little as 5 days of space flight and this period of unloading renders muscles susceptible to reloading injury [84].

Muscular atrophy, elicited by either the reduction in contractile activity, a shortened working range, and unloaded contractions, is the appropriate response for efficient functioning at both low workloads [85] and low tension. Yet, the loss of muscle mass presents a problem when, for example, astronauts return abruptly to Earth (i.e., a 1-gravity environment) and rely upon the microgravity weakened muscles to contend with heavy workloads [85]. The stress of returning to gravity loading reveals serious impairments to the normal functioning of skeletal muscle when weight bearing activities are required with structurally weaker (i.e., atrophied) myofibrillar units [15]. Muscle fibers are damaged when mechanical

loads are reestablished due to the inability of these skeletal muscles to support body weight and/or contractile activities similar to that prior to unloading.

The damage to structurally weaker skeletal muscles following reloading is similar to damage observed following unaccustomed eccentric exercise [35, 37], with one major difference. The eccentric damage to normal muscle requires high intensity exercise whereas the damage to atrophic muscles can occur following simple voluntary movements [35]. In other words, muscles accustomed to normal activity injure only when subjected to higher unaccustomed exercise. Yet, muscles that have been accustomed to low activity (e.g., during bed rest, limb casting and space flight) will injure when normal activities are presented (e.g., during reloading or a return to Earth). Numerous similarities between unloaded/reloaded sarcomere lesions and those lesions related with delayed-onset muscle soreness (DOMS) following unaccustomed eccentric exercise have been noted [36, 86-88].

The literature [50, 51] suggests that an initial bout of unaccustomed eccentric exercise alleviates muscle damage following a second bout. This phenomenon is known as the repeated bout effect (RBE). For example, Schwane et al. (1983) demonstrated an attenuated injury response in a subsequent downhill exercise bout following a conditioning bout of downhill exercise in rodents. Further, the same protective effect is seen in human studies. For example, Clarkson and Tremblay (1988) showed decreased serum creatine kinase activity, reduced muscle soreness and pain, and attenuated strength loss following the second eccentric exercise bout. While this protective effect has been recognized for four

decades, the exact mechanism behind this action is still debated. To date, three major theories have been proposed: (1) the neural theory; (2) the connective tissue theory; and (3) the cellular theory [89]. Other possible mechanisms include (4) adaptations in excitation-contraction (E-C) coupling [57, 58] and (5) adaptations in the inflammatory response to damage [59].

2.2 Repeated Bout Effect

The possible mechanisms for the repeated bout effect are summarized as follows: (1) the neural theory proposes that exercise-induced muscle damage occurs in a fairly small number of type II muscle fibers. During a subsequent bout of exercise, the pattern of muscle fiber recruitment is altered and a larger number of muscle fibers are activated. Since more fibers are recruited, the relative stress to each individual fiber is lessened and injury is reduced during subsequent bouts of exercise [90]. (2) The connective tissue theory proposes that in order to provide more protection during the stress of exercise, connective tissue increases in response to the muscle injury that occurred during the initial bout [90]. (3) The cellular theory proposes that in response to the exercise-induced damage, new proteins (e.g., stress proteins, cytoskeletal proteins, etc.) are synthesized to enhance the integrity of the muscle fiber. Thus, less strain is placed on the muscle fiber and it is protected from a subsequent bout of the exercise [90]. (4) Impairment of the calcium-mediated E-C coupling has been proposed to explain the decreased force production seen following eccentric contractions [57]. This could result from either impaired calcium release or sensitivity [89] subsequent to

any myofibrillar damage. Therefore, an adaptation to these processes lessens damage following the second bout. (5) Lastly, a decline in the inflammatory response to repeated exercise has been proposed as a contributing protective mechanism. A blunted immune response to the repeated bout would reduce the magnitude of edema within the cell. However, it is unclear whether reduced inflammation is associated with a blunted immune response subsequent to tissue damage or a lack of tissue damage following the exercise bout [89]. Adaptations in the inflammatory response could account for the lack of further damage when repeated bouts are performed prior to full muscle recovery. [52, 91].

To date, it is unknown which theory best explains the RBE. No one theory can explain all the various observations that occur under these circumstances. Therefore, it is likely that this phenomenon is a result of intricate interactions between any or all of the proposed neural, connective tissue, cellular and other factors seen in response to exercise-induced injury [89].

Regardless of the inability to accurately determine the mechanisms of the RBE, exercise that causes skeletal muscle damage results in prompt adaptations within the muscle cell. The muscle then becomes more resistant to the damaging effects of a second bout of the same exercise [50]. Generally, it has been recognized in the literature that exercise-induced muscle damage is particularly associated with eccentric contractions [42, 92, 93]. Investigators have suggested that sarcomeres have a physical threshold to damage, which is surpassed with

unaccustomed eccentric exercise [94]. Once this physical threshold is surpassed, sarcomeres will injure with continued contractions.

Eccentric contractions are distinguished from isometric and concentric contractions in that during contraction they induce greater force production and longer muscle lengths [92]. The force generated by a muscle during eccentric contractions is less than that of the opposing load [89], and therefore the muscle is actively lengthening during force production. Tension generation can be described in two phases: 1) the muscle contracts to produce tension and 2) while still generating additional tension, the muscle is passively stretched by the external load [93]. Therefore, sarcomere length for any given muscle length is increased during lengthening contractions and the sarcomeres move toward the descending limb of the force-length relationship [95]. From a mechanical standpoint, such high force production that occurs while the muscle is lengthening may attribute to the increased damage associated with these types of contractions [92]. Further, Morgan (1990) suggests that the descending limb of the force-length relationship is unstable. Hence, this instability leads to increased susceptibility of sarcomeres to damage when contractions are performed eccentrically [96].

Damage to reloaded skeletal muscle is morphologically comparable to damage observed after unaccustomed eccentric exercise. Therefore, perhaps the mechanisms of damage are similar. Thus, initial bouts of eccentric exercise prior to skeletal muscle unloading may demonstrate the same protective effects as observed in previous investigations; where initial eccentric exercise protected

against muscle damage during the second bout of eccentric exercise [50, 51]. However, in order to determine if eccentric exercise protects against reloading damage, muscles must undergo a period of unloading sufficient enough to elicit atrophy. Skeletal muscle atrophy will result in the weakening of the contractile unit. Then, muscle damage will ensue when the muscle is subjected once again to mechanical loads (i.e., reloading) similar to those prior to unloading.

Several animal models have been developed that are capable of experimentally inducing muscle atrophy. These models include: space flight [8, 45, 48, 77, 84, 97-103], immobilization [3], spinal cord transection [104, 105] [60, 61], denervation [104, 106] and hindlimb suspension unloading [10, 22, 28, 32, 70, 107-109]. Limb immobilization has been used for decades to protect bones and injured tissues from repeated injury. As a consequence of muscle disuse during immobilization, muscle wasting also occurs [63]. Spinal cord transection and denervation are similar models since they induce either an upper or lower motor neuron lesion [63]. Disruption of the lower motor neuron is seen in denervation models whereby the upper motor neuron is disrupted in spinal cord transection. In both these circumstances, electrical signals serving muscle fibers are attenuated and/or silenced. Therefore muscle contraction and utilization are decreased and muscle fiber atrophy occurs [105].

2.3 Hindlimb Suspension Unloading

In 1979, a new model to study muscle fiber atrophy was developed [62]. In cooperation with the National Aeronautics and Space Administration, Emily Morey-

Holton designed the hindlimb suspension unloading model. It had been documented that astronauts return to Earth physically weaker, showing evidence of skeletal muscle atrophy and DOMS [63]. Due to the infeasibility of conducting research in space, this model was developed as a means to simulate a weightless environment [63] and thereby conduct Earth-based investigations on the alterations that occur in organ systems under such conditions.

The hindlimb suspension unloading (HSU) model involves harnessing rats to elevate the hind limbs and removing the weight bearing function of the suspended muscles [63]. The rats are able to navigate about the cage by using their forelimbs. Early experimental results utilizing this technique demonstrated physiological responses similar to those documented in space flight (i.e., bone mineral loss, interstitial fluid shifts, and muscle atrophy) [63, 79, 110]. Further, the physiological adaptations that occur with hindlimb suspension unloading are similar to those observed with other decreased-use models (i.e., spinal cord transection, denervation and immobilization) [60, 61, 105, 111] and thereby give this model duel application. Hence, not only can physiological systems be studied under conditions of simulated microgravity, but the alterations that occur can also be compared to other decreased-use models. Therefore, this model provides an opportunity to study a decreased-use model where motor neurons are intact and muscle tension extremely low [63]. Thus, physiological alterations can be induced in particular skeletal muscles, as well as entire organ systems, without the need for invasive procedures.

Physiological alterations that occur in the muscular system as a result of unloading have been elucidated by the use of the HSU model (and other decreased-use models). Skeletal muscle adaptations to unloading have been differentiated into primary (induced by unloaded environments) and secondary (induced by reloading) changes [85]. Muscle atrophy and a shift in the metabolic properties of skeletal muscles are among those considered primary alterations. Among the secondary adaptations are muscle weakness, fatigue, DOMS, incoordination, and muscle damage [15, 35, 112].

2.4 Primary Adaptations

Tissue removal preceding muscle reloading in HSU investigations has uncovered several primary adaptations to unloading [8, 28, 35]. These effects are differentiated from secondary alterations that appear in muscle tissue obtained after release from HSU and reestablishment of weight-bearing activities, usually hours to days later [84, 113]. For example, one primary adaptation is muscle atrophy [45, 70, 80, 114]. Following 7 d of HSU, soleus and gastrocnemius muscles were 42% and 28% smaller than control muscles [70]. Similarly, following 7 d of HSU in rabbits, soleus muscle length was decreased by 35% [32]. Further, subsequent to 7 d of space flight and 12-16 h post flight, rat SOL cross sectional area (CSA) declined by 29.3% and EDL CSA declined by 23.2% [19]. Differential rates of atrophy were observed in rat muscles following 22 d of space flight and were first reported by Ilyina-Kakueva and co-workers (1976). Post flight, the average weight of the soleus muscle declined by 32% and the extensor digitorum

longus declined by 12% when compared to control muscles. Further, the decline in weight of the gastrocnemius and quadriceps were non-significant and the weight of the biceps did not change. Interestingly, the magnitude of decline in the soleus muscle varied between and did not occur in all animals. The investigators concluded that some animal's muscles are more susceptible to atrophy than others under conditions of unloading.

Certain characteristics determine whether skeletal muscles will be more or less susceptible to unloading atrophy. Generally, leg extensor muscles (e.g., soleus and adductor longus) serve to lift the body against gravity and are called antigravity muscles [15]. Antigravity muscles are characterized by low-myosin ATPase activity; slow times to contraction, contain higher percents of slow-twitch fibers and have high levels of oxidative metabolism [15]. On the other hand, flexor muscles (e.g., tibialis anterior and extensor digitorum longus) are non-weight bearing, contain a preponderance of fast-twitch fibers, contract rapidly, and are equipped with enzymes specialized for anaerobic glycolysis [15]. Typically, these muscles do not have an antigravity function and are periodically utilized during short-term activity and therefore are less affected by prolonged periods of unloading. In summary, fibers that cross a single joint, have an antigravity function and contain a high percent of slow fibers are most susceptible to muscle atrophy induced by unloading [63, 79, 102, 103, 109, 110, 115].

Given the physiological and morphological characteristics of muscles, the magnitude of unloading-induced atrophy can be predicted. Generalizations

concerning unloading atrophy have been developed and a hierarchy of muscles most susceptible to the least susceptible established. As stated previously, the hierarchy is as follows: soleus (SOL) > gastrocnemius and plantaris > extensor digitorum longus (EDL) and tibialis anterior [64]. Due to the SOL muscle's increased susceptibility to atrophy, this muscle is perhaps that most investigated when studying unloading atrophy [19, 45].

The rat SOL muscle is composed of at least 80% type I, slow oxidative (SO) fibers and ~20% type IIa fast oxidative glycolytic (FOG) fibers. The SOL also has an antigravity function and crosses a single joint [63]. In contrast to the SOL, the EDL muscle does not have an antigravity function, and consists predominantly (~90%) of fast twitch fibers [63]. Since the rat soleus (predominantly slow-twitch) and EDL (predominantly fast-twitch) muscles consists primarily of one fiber type, these muscles have been studied extensively to elucidate any fiber type specific alterations that may occur during unloading. The SOL and EDL muscles will be the focus of this investigation. Further, the SOL was chosen because during eccentrically biased activities (downhill exercise), this muscle undergoes a preponderance of eccentric contractions [37]. At any rate, muscle atrophy elicited by unloading is thought to be the result of decreased fiber cross-sectional area (CSA) as opposed to cellular death [116] and therefore nearly all of the primary alterations represent simple deconditioning (i.e., a reduction in muscle size) without pathology [15].

2.5 Secondary Alterations

Following HSU and/or space flight and 0 h of muscular reloading, investigators have shown muscular atrophy, decreases in muscle wet weight, increased expression of the fast myosin type and myofiber necrosis [28]. Rat SOL muscles examined from the Cosmos 1887 biosatellite (12.5 days) showed these very trends: (1) a 15% decline in wet weight, (2) a 40% decline in myofiber cross-sectional area (CSA), and (3) an increased expression of fast myosin properties [28]. Yet, subsequent to muscular reloading, further alterations in skeletal muscle have been observed within 5 h of muscular reloading and include increases in muscle wet weight and CSA [117]. Muscular unloading results in a decline in muscle wet weight in comparison to controls. Yet, the decline in muscle wet weight becomes less evident as the duration of reloading increases. For example, following 7d of HSU, SOL wet weight was increased significantly in the 1 d and 2 d reloaded group as opposed to the 0 d reloaded group (100 mg, 100 mg vs. 70 mg, respectively) [21]. Similarly, subsequent to 12.5 d of HSU, muscle CSA decreased by 62% compared to control muscle CSA. However, during 12, 24, and 48 h of reloading, the decline in CSA was less drastic (54%, 53%, and 55%, respectively). [28]. Light microscopic techniques revealed an increase in the number of damaged fibers, interstitial edema and mononuclear cell infiltration [28]. In other words, the influx of interstitial fluid and mononuclear cells into the muscle cell increased muscle wet weight. Further, these rats exhibited severe sarcomere fragmentation and Z-line streaming when viewed with an electron microscope [35]. Z-line

streaming is defined as the occurrence of Z-line-like material that protrudes from the Z-lines into the sarcomere to varying degrees [35].

Additionally, similar degenerative alterations (i.e., an increase in damaged fibers, interstitial edema and mononuclear cell infiltration) were observed after Cosmos flights 605 and 690 in both muscles biopsied within 2-3 hours after space flight, and in muscles biopsied 2 days later [28]. A common occurrence among these investigations is that the muscles were examined subsequent to reentry and reloading of the muscles. In other words, the muscles had been subjected to gravity and weight bearing activities prior to morphological and/or physiological examination. Therefore, consistencies in muscle morphology between these results led investigators to question whether the pathologies observed were induced by unloading or caused by re-exposure to weight bearing.

Twelve and one half days of HSU and 0-hour reloading (sacrificed immediately after suspension) demonstrated changes similar to the typical space flight induced alterations: decreased wet weights, decreased myofiber CSA, and increased expression of fast myosin. However, muscles examined morphologically during periods of reloading (12-48 hours after HSU) showed increased soleus wet weights, interstitial edema, macrophage activation and monocyte infiltration [28]. This investigation proved that “the degree of and type of muscle degenerative changes observed post-flight depends upon the duration of gravity readaptation before biopsy and not solely on exposure to microgravity.” [28].

Similarly, Anzil et al. (1991) observed muscle degeneration after 7 days of HSU and 2 min of reloading in rabbit SOL muscles. These investigators characterized the disruption as: 1) fibers that are wavy in contour, 2) loss of Z-line(s), 3) focal disorder of the myofilaments (i.e., actin and myosin) and 4) mitochondrial depletion [32]. Vijayan et al. (1998) observed focal disorder of the myofilaments and similar pathologies at the light microscopic level. Rat adductor longus (AL) muscles exposed to 14 days of HSU and a subsequent 12-14 h period of reloading revealed myofibril lesions as pale patches in AL semi-thin sections [24]. This abnormal morphology indicated A-band disruption and also revealed the preferential damage of slow-fibers, accounting for 92 $\pm$ 2% of the affected population. Similar conclusions were reported in rat SOL muscles of the STS-58 mission (14 days) and in vastus medialis muscles in both HSU and on the Cosmos 2044 (both 14 days) [24]. The authors attributed the damage to voluntary movement and motor unit recruitment during reloading [24].

Following the primary adaptations that occur in muscle fibers (i.e., atrophy and decreased CSA), damage occurs when the muscle fiber contracts during reloading [24]. For example, adductor longus muscles examined 8-11 h post-flight (Cosmos 2044) exhibited damaged sarcomeres similar to human muscles exposed to eccentric contractions [35]. The sarcomere damage observed in adductor longus (AL) muscles following space flight [35] were comparable to those reported by Fridén et al. (1980). Subsequent to eccentric exercise, muscle abnormalities at the cellular level included focal disturbances of the cross-striated

banding pattern. Ultrastructurally, these muscle disturbances originated from the myofibrillar Z-lines, which were markedly wide, smearing, and in some instances completely disrupted [43]. Further damage was noted to sarcomeres adjacent to the above-mentioned Z-line. These sarcomeres were either supercontracted or disorganized and out of register. When adjacent sarcomeres lose register, the appearance of the muscle fiber follows a zigzag pattern [118].

Similarly, pilot work in our laboratory revealed varying degrees of Z-line damage (e.g., wavy in contour, missing, fragmented and streaming) in rats subjected to 7 d of HSU and a 16-19 h reloading period [30]. Therefore, similar morphological changes (i.e., disrupted striated banding patterns, Z-line streaming, etc.) are observed following both unaccustomed eccentric exercise and hindlimb suspension unloading and subsequent reloading. Fridén et al. (1980) concluded that during overload, the Z-lines are the weak link in the myofibrillar contractile chain. In summary, muscle damage is a secondary alteration that results from unloading atrophy and the reestablishment of weight bearing activities during reloading. The morphological changes share similar characteristics to those observed following unaccustomed exercise.

2.6 Quantifying Muscle Damage

Whether myofibrillar disruption is caused by reloading or unaccustomed eccentric exercise, skeletal muscles have a tremendous ability to repair themselves [119]. Normal muscle fibers (those not hindered by a genetic disease) swiftly repair sarcomere lesions by Z-line-like patching [8, 15, 21, 29, 35, 120]. Z-

line-like patching is the lateral protrusion of Z-line-like material from the Z-line and into the sarcomere [35]. Hypothesized to represent structural repair, this material serves to transmit tension across the damaged sarcomere so upon further contraction, the sarcomere will not be pulled apart at the lesion [35].

Further evidence of structural repair can be observed by interstitial edema and infiltration of the muscle cell by monocytes and macrophages [37]. Lieber et al. (1994) hypothesized that muscle injury led to the extravasation of monocytes and leukocytes from the bloodstream and the infiltration of these cells into the damaged tissue [121]. Investigators have utilized the presence of monocytes, macrophages, interstitial edema, disrupted striated pattern (via light microscopy) [8], and Z-line streaming and sarcomere disruption (via electron microscopy) [43] as a tool for identifying sarcomere damage. Further, enzymatic quantification of muscle damage has been used extensively in the literature [37, 50, 51]. The release of several muscle-related enzymes (e.g., creatine kinase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase) after damage or injury has been quantified through blood serum [37, 50] or muscle homogenates [31]. Skeletal muscle glucose-6-phosphate dehydrogenase (G-6-PDH) activity has been previously shown to be associated with muscle damage [37]. G-6-PDH activity increased following HSU and reloading in rat skeletal muscle [31] and corresponded to increased morphological damage. Many investigators have consistently recognized an increased activity of G-6-PDH during muscle regeneration [122, 123].

Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme in the Pentose Phosphate Pathway (PPP) and this pathway provides reducing equivalents for lipid synthesis and pentoses for nucleic acid synthesis [123]. The PPP pathway is illustrated in Figure 2.1. The production of ribose-5-phosphate (one by-product of the pathway) is especially important following muscular damage or trauma. Since ribose-5-phosphate is essential for the production of nucleotides and nucleic acids (i.e., CoA, ATP, NAD, NADP, FAD, RNA and DNA) [124], it is logical that the activities of this pathway increase following injury and during repair.

Armstrong et al. (1983) found that downhill running in rats induced the greatest increase in G-6-PDH activity as opposed to either uphill or level running. No changes in G-6-PDH activity were observed during level running. However, G-6-PDH activity increased significantly at 72 h ($^{+}$161% in the medial head and $^{+}$61% in the lateral head of the triceps brachii) following downhill running. G-6-PDH activity also increased significantly at 24 h ($^{+}$31% in the medial head of the triceps brachii) in the uphill runners [37]. In another laboratory, the activity of the PPP was shown to increase by 50% in rat muscle interstitium following 3.5 – 4 h of walking on a motorized treadmill [73]. Activity of the PPP peaked significantly ($p < 0.05$) above both the control group and 0 h post-exercise. Therefore, measuring skeletal muscle G-6-PDH activity following damage will provide an enzymatic means of quantifying degenerative processes [37] taking place within the muscle cell.

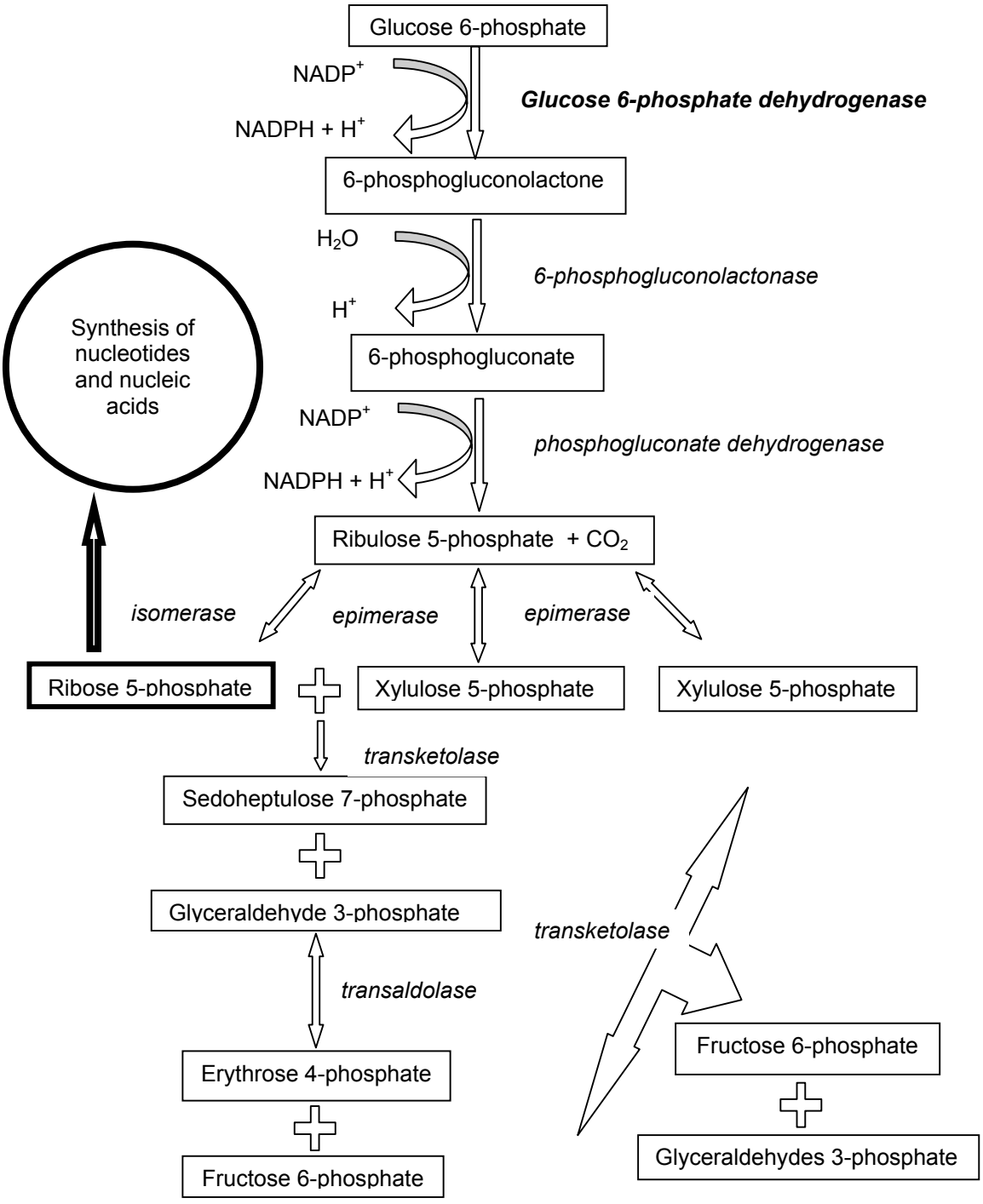


Figure 2.1. The Pentose Phosphate Pathway.

The production of Ribose 5-phosphate allows for the production of nucleic acids and nucleotides. Glucose 6-phosphate dehydrogenase is the rate-limiting enzyme of this pathway. Substrates and products are shown in blocks. Enzymes are italized.

2.7 Exercise Countermeasures

Astronauts are one subgroup of the population often exposed to periods of unloading when traveling in weightless environments. Astronauts have had to rely upon self-directed and voluntary exercise activities to maintain fitness levels adequate enough for the rigors of space flight [125]. This lack of a coordinated exercise program has led to astronauts participating in unsupervised gym activities, competitive athletics (e.g., basketball) and running for preflight aerobic and strength conditioning [125]. Due to self-regulated conditioning, Jennings and Bagian have reported an unusually elevated number of injuries and orthopedic surgeries among this extremely small population and suggest that employment of a full-time training and conditioning staff for pre-, in- and post-flight may be appropriate. However, because astronauts have not followed concerted training programs, the most appropriate exercise prescription for pre-, in- and post-flight conditioning is still unresolved.

Some investigators have suggested that pre-flight endurance training programs may adversely decreased blood pressure and orthostatic tolerance following flight [126-128]. Investigators have reported greater incidences of orthostatic intolerance in the highly aerobically trained (VO_{2max}) as opposed to humans or rats with lower aerobic capacities [127, 129-133]. For example, after 28 d of bed rest, a 22% decline in VO_{2max} was observed in an individual with an initial VO_{2max} of 4.15 liters$\cdot$min^{-1} as opposed to a 13% reduction observed in an individual with an initial VO_{2max} of 3.54 liters$\cdot$min^{-1} [134]. Orthostatic intolerance, defined as the

inability to maintain adequate blood pressure while in an upright posture [15], is seen in ~2/3 of astronauts tested during early post flight [135], in the elderly population and in individuals subjected to prolonged of bed rest. Since aerobic conditioning has been the most prevalent form of exercise training before and during space flight, Tipton (1983) speculated that such protocols accentuated orthostatic intolerance. Furthermore, data from previous NASA missions has demonstrated that only 50% of maximum oxygen consumption is needed for the most demanding of task during flight [136].

Since pre-flight endurance training may adversely affect orthostatic tolerance and VO_{2max} following flight and since high levels of cardiovascular conditioning are unnecessary during flight, exercise protocols should not be directed entirely towards aerobic conditioning. Even though muscle weakness, damage and incoordination continues to be a problem following unloading [137], the impact of training status on reloaded muscle has not been investigated. Unlike reports of cardiovascular training status, it is unknown whether resistance training will negatively or positively impact atrophy during unloading and/or impact skeletal muscle function and morphology during reloading. Therefore, it is appropriate to determine the effects of a resistance-training program on reloaded skeletal muscle morphology.

Similar to pre-flight conditioning, in-flight exercise protocols continue to be directed mainly towards cardiorespiratory conditioning, which does little or nothing to alleviate skeletal muscle deconditioning [15]. While some resistance exercise is

conducted in-flight, exercise protocols have not proven totally effective in the prevention of muscular declines [15]. Table 2.1 depicts results from several HSU investigations, which utilized exercise programs that were concurrent with the period of unloading. All studies presented in Table 2.1 were able to attenuate SOL muscle atrophy to some extent, however the resistant training investigations did so in significantly less time than the cardiovascular training protocols. Aerobic exercise protocols designed for astronauts require a significant amount of time daily (up to 2 h/ day) to complete [70] and therefore future exercise protocols should be designed taking time constraints into consideration. To date the most appropriate training protocols (i.e., eccentric vs. concentric, exercise intensity, duration and the number of repetitions) are unknown [138].

Table 2.1. Exercise countermeasures to prevent soleus muscle atrophy.

Treatment	% Decrease from Controls		Min/D	Reference
	SOL			
	HSU	ExHSU		
Running				
20 m/min, +19° grade, 10 min, 4/day	38	2	40	[70]
20 m/min, -19° grade, 10 min, 4/day	38	9	40	[70]
5 m/min, +19° grade, 10 min, 4/day	28	8	40	[74] [70]
Climbing (primarily concentric action)				
1 m at 85° grade, 50% load, 4 x10 reps/day	38	4	~6	[70]
1 m at 85° grade, 75% load, 2 x 10 reps/day	31	14	~3	[70]
1 m at 85° grade, 75% load, 4 x 8 reps/day	32	8	~6	[70]

HSU, hindlimb suspension unloading; ExHSU, hindlimb suspension unloading + exercise.

During a HSU study, Herbert et al. (1988) was able to attenuate atrophy in rat SOL muscles when initiating a resistance exercise protocol that required only 6 $min \cdot day^{-1}$ to complete. While this investigation was able to attenuate certain primary alterations (i.e., muscle atrophy), secondary alterations (e.g., myofibrillar damage) were not examined to assess how such an exercise protocol may affect them during reloading. Skeletal muscle adaptations following disuse have received considerably less attention than those adaptations that occur during unloading [139]. In the Herbert et al. (1988) investigation, the rats were placed on an intermittent high-load (75% body weight) training program where they climbed a 1-meter grid (~85°) for 8 repetitions (4 sets daily). Even though this protocol attenuated muscle atrophy, it could be argued that climbing the grid (a primarily concentric activity) may not be the most effective mode for protection against eccentric-like contraction-induced damage. However, having the rats descend the grid (i.e., a predominantly eccentric activity) may be more effective in the attenuation of reloading muscular damage.

Further, the exercise interventions that are presented in Table 2.1 were conducted concurrent with the suspension period. A training program prior to unloading may be more effective in not only decreasing the muscle's susceptibility to injury (repeated bout effect), but also may give the muscle a greater reserve from which to lose muscle mass during the unloading period and protect against unloading atrophy. The design of the present investigation permitted the

evaluation of whether or not a prior eccentric training protocol would attenuate reloading damage.

2.8 Summary

As a result of unloading, skeletal muscles atrophy. Due to the inability of atrophied muscle to support body weight and/or contractile activities similar to that prior to unloading, muscle fibers are damaged during reloading. Investigators have utilized the presence of interstitial edema, and disrupted striated patterns (light microscopy) as a tool for identifying sarcomere damage. Further, the release of muscle cell-related enzymes (e.g., glucose-6-phosphate dehydrogenase) after damage or injury has been quantified in blood serum or muscle homogenates.

Investigators have noted similar damage characteristics (i.e., interstitial edema, disrupted striated pattern and increased activity of glucose-6-phosphate dehydrogenase) in sarcomere lesions of unloaded/reloaded muscle and in lesions related with DOMS following unaccustomed eccentric exercise. Initial bouts of eccentric exercise prior to skeletal muscle unloading may demonstrate the same repeated bout effect reported following a second bout of eccentric exercise. However, in order to test this hypothesis, muscle atrophy must be induced. Therefore, muscle damage will ensue when weight-bearing activities are reestablished.

The hindlimb suspension-unloading model, designed to study muscle atrophy, has provided invaluable insight on the physiological adaptations that occur with unloading. Skeletal muscle adaptations have been differentiated into

primary and secondary changes. In other words, muscle atrophy is induced by the period of unloading (a primary change) and reloading induces muscle damage (a secondary change). Further, certain characteristics predetermine which muscles will be most susceptible to unloading atrophy. Generally, leg extensor muscles (e.g., SOL) have an antigravity function, contain a high percent of slow-twitch fibers and are most susceptible to unloading atrophy. In contrast, flexor muscles (e.g., EDL) are non-weight bearing, contain a preponderance of fast-twitch fibers and are less affected by periods of unloading.

Astronauts are one subgroup of the population affected by periods of unloading. An unusually high incidence of injuries and orthopedic surgeries has been reported in this small population. The most appropriate exercise prescription for pre-, in- and post-flight conditioning is unresolved. Investigators suggest that pre-flight endurance training programs adversely affect blood pressure and orthostatic tolerance following flight. Further, only 50% of maximum oxygen consumption is needed during flight. Yet, exercise training continues to focus mainly on cardiovascular conditioning. Moreover, the impact of resistance training on skeletal muscle atrophy during unloading and/or impact skeletal muscle function and morphology during reloading is unknown. Several investigations have utilized exercise programs that were done concurrent with the unloading period. Further, these investigations utilized exercise training that required a preponderance of concentric muscle action, even though atrophied muscles are most susceptible to damage induced by eccentric actions. Therefore, an eccentric

training program prior to unloading may attenuate reloading damage (repeated bout effect).

CHAPTER 3. METHODS

3.1 Experimental Protocol

The Louisiana State University's Institutional Animal Care and Use Committee (IACUC) reviewed and approved all experimental procedures utilized in this investigation. Forty-eight female Sprague Dawley rats (6-9 weeks) were obtained from the Louisiana State University School of Veterinary Medicine (Division of Laboratory Animal Medicine, Baton Rouge, LA). Due to the large number of rats in this experiment, the study was conducted in three stages. Each of the three stages consisted of 16 rats divided into 4 experimental groups. At the beginning of each stage, the rats were randomly assigned but stratified by age and weight so that each group had equal representation in each stage. Therefore, overall mean body weight and age did not differ between the experimental groups. Rats were assigned to one of the following four groups: Exercise + Hindlimb Suspension Unloading (ExHSU, n=12); Hindlimb Suspension Unloading (HSU; n=12); Exercise (Ex; n=12), and Control (C; n=12). Body weight was monitored 3 days/week (Monday, Wednesday and Friday). During the hindlimb suspension unloading phase, body weight for suspended animals was measured daily to assure appropriate physiological responses and animal safety. The rats were housed in groups of 2-4 animals per plastic cage (20 x 22 x 42.5 cm) in a temperature (21-22°C) controlled environment with a 12:12 h light-dark cycle. Access to food (standard rat chow) and tap water was provided *ad libitum*

throughout the duration of the experiment. The timeline for the experimental protocol is described in Table 3.1.

Table 3.1. Timeline for experimental protocol.

WEEK 1							
2-3 days exercise familiarization	Day 1 10% BW	Day 2 20% BW	Day 3 30% BW	Day 4 40% BW	Day 5 50% BW	Day 6 Rest	Day 7 Rest
Week 2							
Day 8 50 % BW	Day 9 50 % BW	Day 10 50 % BW	Day 11 50 % BW	Day 12 50 % BW	Day 13 Rest	Day 14 Rest	
Week 3							
Day 15 Rest	Day 16 HSU	Day 17 HSU	Day 18 HSU	Day 19 HSU	Day 20 HSU	Day 21 HSU	Day 22 HSU
Week 4							
Day 22 Reloading (16-19 h)	Day 23 sacrifice						

BW, body weight; HSU, hindlimb suspension unloading.

3.2 Exercise Protocol

During the exercise protocol, the exercise groups (ExHSU & Ex) descended a 1-m grid inclined at a ~45° angle (Appendix I, Figure I.7). Following 2-3 repetitions of warm-up (rats descended the grid with no additional weight), two sets of 10 repetitions were completed with a 3 min rest period in between set. The exercise protocol was completed 5 d per week for 2.5 weeks. During the first 2-3 days of the exercise protocol, the rats exercised without added resistance during both the warm-up and exercise repetitions (familiarization period). Once accustomed to the exercise protocol, the rats carried a specific resistance down the incline. A small plastic baggy filled with BB's (Daisy Manufacturing Co., Rogers, AR) served as the resistance and was attached to the animal's back with

Co-Flex (Andover Salisbury, MA) and medical tape. Following the familiarization period, resistance (equaling 10% body weight) was added to the exercise protocol. Each day thereafter, weight was increased daily (10% increments) until the resistance equaled 50% of the animal's body weight. At this point, exercise continued at this set resistance for the remaining 5 days of the exercise protocol. Body weight was measured throughout the experiment and the resistance adjusted accordingly.

3.3 Hindlimb Suspension Unloading (HSU)

Three days following the cessation of exercise, the ExHSU and HSU groups were suspended with medical tape and Co-Flex. Animals were anesthetized (1 liter/minute oxygen and 1-3% to effect for isoflurane) to allow placement of the harness and tail suspension apparatus. The base of the tail was washed, dried and coated with Compound Benzion tincture (Perrigo, Allegan, MI) to inhibit slippage of the tail apparatus during suspension and to prevent the adhesive tape from irritating the skin. The Tincture of Benzion was allowed to dry slightly before applying strips of Co-flex around the tail's base. Medical tape was used to secure the Co-flex to the tail. Next, Co-flex (secured with medical tape) was wrapped in a figure eight fashion around the animal's torso and fore limbs so that it resembled a harness. The harness and the tail apparatus were secured with string and fish swivels to a guiding rod at the top of the cage, so that the animals could be lifted in a head-down tilt position (24 hr/day), unloading the hind limbs. The hind limbs dangled down freely but could not touch the cage floor nor touch

the sides of the cage. The hindlimb suspension model can be viewed in Appendix I (Figures I.1 and I.2). Rats were monitored 2-3 times/day and harnesses adjusted to prevent weight bearing of the hindlimb muscles. Also, these daily checks ensured that the animal's tail did not discolor and that the tail apparatus was not constrictive to blood flow. The animals were able to sleep on their chests and fore limbs. The hindlimb suspension unloading phase of the experiment continued for 7 d and the animals were then released and allowed to assume a normal posture. Sacrifice time occurred between 16-19 h of reloading.

3.4 Tissue Acquisition

The animals were euthanized (CO_2 gas) and the following muscle samples obtained: left and right SOL and right EDL muscles. Adrenal glands were removed, trimmed of fat, weighed and discarded. All tissues were weighed using a Mettler scale (model: AE50). The right SOL and EDL muscles were weighed wet upon dissection and frozen. The muscles were then freeze-dried (Virtis Benchtop 3L Research Freeze Dryer, Gardiner, NY) at a later date and dry weights obtained. Tibias were removed to assess the maturation rate of the animals during the experiment [70, 140]. The right tibia was removed, stored in saline and frozen. At later date, the bone was cleaned of muscle and connective tissue. Tibia length (mm) and bone mineral content was measured using a pDEXA Sabre bone densitometer (Norland Stratec) provided by the Human Ecology Department at Louisiana State University.

3.5 Enzymatic Assay

A portion (~50 mg) of the right SOL (dried) and right EDL (dried) was prepared and assayed (in duplicate) for glucose-6-phosphate dehydrogenase according to Wagner et al. (1978) and the mean rate of change (8 minutes of activity) compared between groups. Muscle samples were first homogenized (1:10) in 50 mM Tris and 0.5 mM Ditiothreitol (DTT). Calibration standards (pH 4, pH 7 and pH 10) were used to adjust the homogenate to a pH of 7.6 using a Sargent-Welch pH meter (model: pH 8200). The homogenate was then centrifuged (CRU-5000 centrifuge, Damon/IEC Division) at a temperature of 4°C and at 3500 revolutions per minute for 60 minutes. The resulting supernatant was stored frozen until analysis. On the day of analysis, the assay reagent was mixed fresh and consisted of the following: 50.0 mM Tris, 3.0 mM glucose-6-phosphate, and 0.5 mM NADP. The assay reagent was analyzed using a Hitachi spectrophotometer (model 100-40, Tokyo, Japan) set to a wavelength of 340 nm. The reagent (0.9 ml) was added to a 1.4 ml cuvette, mixed and the optical density (O.D.) read for 2 min. The thawed muscle homogenate (0.1 ml) was added, mixed and the O.D. recorded every 30 seconds for 8 minutes and the average O.D. was calculated for each minute. Each muscle sample was read in duplicate and the mean taken for statistical analysis. The following steps and equations were used to elicit the final enzyme activity in $\mu mole \cdot g^{-1} \cdot min^{-1}$ and an example of these calculations are provided:

1) Muscle weight was measured in grams and converted to mg.

0.054 g· 1 ml^{-1} of tissue was converted to 54 mg·1 ml^{-1}

2) Determine the amount of tissue that would be present in .1 ml (cuvette volume). Solve for x:

54 mg / 1 ml = x / .1ml

1ml x = 5.4 mg·ml^{-1}

x = 5.4 mg

3) Determine the enzymatic activity in the muscle homogenate. Solve for the rate of change observed with the spectrophotometer:

Rate of change = final O.D. - initial O.D. / # of minutes.

Mean O.D. = .26 - .24 = .02 / 8 = .0025 O.D.· min^{-1}

4) Mean enzyme activity was multiplied by the amount of enzyme present in the homogenate;

= .0025 O.D. ·min^{-1} x .0054 g = .0000135 g·min^{-1}

5) Enzyme activity (g·min^{-1}) was divided by the extinction coefficient (6.22) to obtain the activity in mole·g^{-1}·min^{-1}.

= .0000135 g·min^{-1} / 6.22

= .00000217 mole·$g^{-1}$$min^{-1}$

6) Convert to μmole·$g^{-1}$$min^{-1}$:

= .00000217 mole·$g^{-1}$$min^{-1}$ x 10^6 = 2.17 μmole·$g^{-1}$$min^{-1}$

3.6 Light Microscopy

Following dissection and weighing, the left SOL were stapled to index cards to maintain resting lengths during fixation. The tissues were bisected with one-half

of the muscle being used to obtain a cross-section of the midbelly and the remaining half being used for longitudinal sections. The tissue was processed according to accepted histological procedures (provided by the Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA) and the cross- and longitudinal sections were stained with phosphotungstic acid-hematoxylin (PTAH). Reagents for the staining procedure include the following: 1) Zenker's solution, 2) alcoholic Iodine solution, 3) 5% sodium thiosulfate (Hypo), 4) 0.25% potassium permanganate solution, 5) 5% oxalic acid solution, 6) phosphotungstic acid-hematoxylin solution (PTAH) (pre-heated to 55-60°C in conventional oven).

The tissue was processed according to the following protocol. Tissue from the left SOL were immediately fixed in 10% non-buffered formalin and placed in paraffin. The tissue was then cut on a microtome (3 μm) and placed on slides. The slides were deparaffinized and hydrated in distilled water and placed in acidified Zenker's solution for 3 hours (55-60°C oven or at room temperature overnight), then rinsed in water. Mercuric chloride crystals were removed from the slides with 0.5 alcoholic iodine (10 minutes) and then the slides were rinsed in tap water. The slides were decolorized with 5% sodium thiosulfate (5 minutes) and rinsed in running tap water for 10 minutes. Following this step, the slides were placed in 0.25% potassium permanganate (5 min), rinsed in tap water, placed in oxalic acid (1 min), and then rinsed again in running tap water (10 min). Finally, the slides were then stained in preheated PTAH solution at 55-60°C for 1 hour (checked

after 30 min) and allowed to cool at room temperature for 30 min. The slides were then dehydrated quickly through 2 changes each of 95% alcohol and absolute alcohol and cleared in 2 changes of xylene and mounted with synthetic mounting medium.

The slides were microscopically examined for inflammatory and myofibrillar changes under a Leitz Laborlux 11 light microscope equipped with an Hitachi Color Camera. The camera was interfaced with a PC containing Scion Image ® software (Scion Corp). Fiber areas were measured with accuracy utilizing a Graphire 2 graphics tablet and pen set (Wacom, Vancouver, WA).

3.7 Analysis

The slides were coded so that the examiner was blind to the experimental group during analysis. The longitudinal and cross sectional images were analyzed once they were captured in a computer file. Fiber areas (μm^2) for 150-myofibers/ cross-sections were measured, the mean taken for each group and compared between groups [141, 142].

Muscle cross sectional area was measured, which was able to distinguish the area occupied by muscle fibers only vs. the area occupied by non-muscle (interstitial) components of the cell (Appendix I, Figures I.3 and I.4). The percent area occupied by interstitial space was calculated (% area of interstitial space = area of interstitial space / total cross sectional area * 100). These values were used to represent interstitial edema. The means were averaged for each group and compared statistically. To assess myofibrillar damage in the longitudinal

sections, damaged fiber area (μm^2) was expressed as a percent of the total fiber area (μm^2) measured. Areas of the fiber were considered damaged if the fiber displayed focal disruptions in the banding pattern [8, 24, 43]. Examples of normal and damaged (i.e., disruption cross-striated banding patterns) SOL muscle can be viewed in Appendix I, Figures I.5 and Figure I.6, respectively. An average percent for each group was calculated for statistical comparison.

3.8 Statistical Analysis

The following variables were compared between groups; changes in mean body weight, SOL wet and dry weight, EDL wet and dry weight, SOL and EDL muscle-weight to body-weight ratios, SOL fiber area to body-weight ratio, adrenal gland weights and adrenal gland to body-weight ratios, glucose-6-phosphate dehydrogenase activity in the SOL and EDL, percent myofibrillar damage, percent interstitial area of the SOL, tibia length and tibia bone mineral content.

A statistical significance of $p \leq 0.10$ was chosen [143]. A one-way analysis of variance with repeated measures was used to assess changes in body weight across time followed by the Student Newman-Keuls (SNK) significant difference test. Group differences were statistically analyzed using one-way analysis of variance (ANOVA). For significant ANOVAs, group differences were tested post hoc by Tukey HSD for equal sized groups and by Student Newman-Keuls (SNK) for unequal sized groups. If homogeneity of variance in a particular variable (i.e., percent myofibrillar damage) was not obtained, groups were analyzed non-parametrically with the Kruskal-Wallis test and followed up with a Dunn's multiple

comparison procedure. Comparison of statistical means via a t-test was not stated in the original statistical design. However, a post analysis t-test was conducted on the percent of myofibrillar damage between the ExHSU and HSU groups, since the proposed hypothesis corresponded with this analysis. Further, statistical correlations and regressions were determined between the percent of myofibrillar damage and fiber area, percent interstitial area and G-6-PDH activity and also between the percent interstitial area and fiber area in the ExHSU and HSU groups. Since significant muscle damage was not induced in the Ex and control groups following the experimental protocol, the correlations were analyzed excluding the data from these two groups. In order to utilize the Pearson Product Moment correlation, the percent myofibrillar damage data was transformed so that it met the criteria set forth for parametric data. The one-way ANOVAs, t-test and correlations were performed with the SPSS v. 10.05 (Chicago, IL)) statistical software program. The Kruskal-Wallis test was performed with the Sigma Stat v. 2.0 (Chicago, IL) statistical software program.

A less conservative significance level of $p \leq 0.10$ was chosen due to the nature of the investigation [143]. The experimental protocol could have been manipulated in several ways. For example, this study utilized just one of many possible exercise protocols (e.g., repetitions and sets), resistance levels (e.g., a load equaling a certain percent of BW), eccentric ramp slopes (i.e., 45° vs. any other angle), and number of training days prior to suspension. Since one or all of these experimental components can be altered in future investigations, a less

rigorous alpha level can aid in determining which perturbation, if any, would have an effect on the desired outcome. Therefore, an alpha level of $p \leq 0.10$ was deemed significant. However, individual *p* values are reported for all experimental variables [143].

CHAPTER 4. RESULTS

4.1 Data Presentation

Individual data for each dependent variable for each rat are listed in Appendices B-F. Data from five rats (#4, #22, #23, #34, #49) were ultimately excluded from analysis of post-experimental variables (adrenal weights, adrenal weight to body-weight ratio, tibia lengths, tibia bone mineral content, body weight at sacrifice and across time, SOL myofibrillar damage, EDL muscle-weight to body-weight ratio, SOL muscle-weight to body-weight ratio, SOL fiber area, SOL fiber area to body-weight ratio, SOL and EDL G-6-PDH activity, SOL and EDL muscle weights) due to incompletion of the suspension period. These rats had either escaped or slipped from the harness and therefore experienced weight bearing on their hind limbs for an unknown period of time before discovery. Thus, the group numbers for these statistical analyses following the suspension period are as follows: ExHSU; n = 9, HSU; n=10, Ex; n= 12, and C; n= 12. Body weight data are listed in Appendix A, muscle weight data are presented in Appendix B, enzymatic activity data are presented in Appendix C, histological data are presented in Appendix D, adrenal weights are presented in Appendix E, and tibia lengths are presented in Appendix F and tibia bone mineral contents are presented in Appendix G.

4.2 Body Weight

Mean body weight (g) data for each group is listed in Table 4.2.1. There were no significant (p = 0.947) group differences in body weight (BW) at the start

of the experimentation nor were there any differences the day prior to suspension (p = 0.889). However, at the time of sacrifice, mean BW was significantly different between groups (Table 4.2.1). The ExHSU and HSU groups had significantly (p = 0.001) lower body weights than both the Ex and Control groups. However, there were no significant differences between the ExHSU and HSU groups.

During the experimentation, no group significantly gained or lost more weight than any other group (p = 0.916). However, there were significant main effects for group (p = 0.000) and time (p = 0.000). The ExHSU group had a significantly lower overall BW than the other groups. As depicted in Figure 4.2.2, pooled body weight data indicates that BW increased during the experiment.

Table 4.2.1. Body weight at sacrifice.

	GROUP				*P* =
	ExHSU	HSU	Ex	Control	
BW (g)	201.7 ± 16.3	207.3 ± 7.6	230.9 ± 18.8 *	226.0 ± 22.1 *	0.001

Values are means ± SD.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise; BW, body weight.
*Denotes significant difference from ExHSU and HSU groups at p = 0.001, Student Newman-Keuls significant difference test.

4.3 Muscle Weights

Muscle weights (SOL and EDL) are presented in Table 4.3.1. EDL wet and dry weights approached statistical significance (p = .168 and p = .110, respectively) between groups. There were significant group differences in soleus wet and dry weights (p = .028 and p = .036, respectively). The ExHSU and HSU

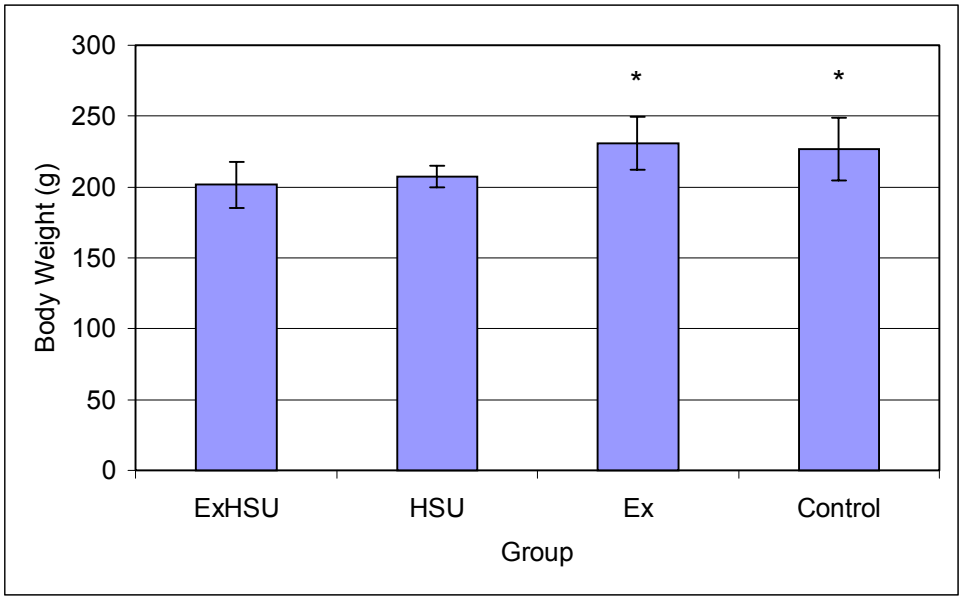


Figure 4.2.1. Body weight at sacrifice.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes significant difference from ExHSU and HSU groups at $p = 0.001$, Student Newman-Keuls significant difference test.

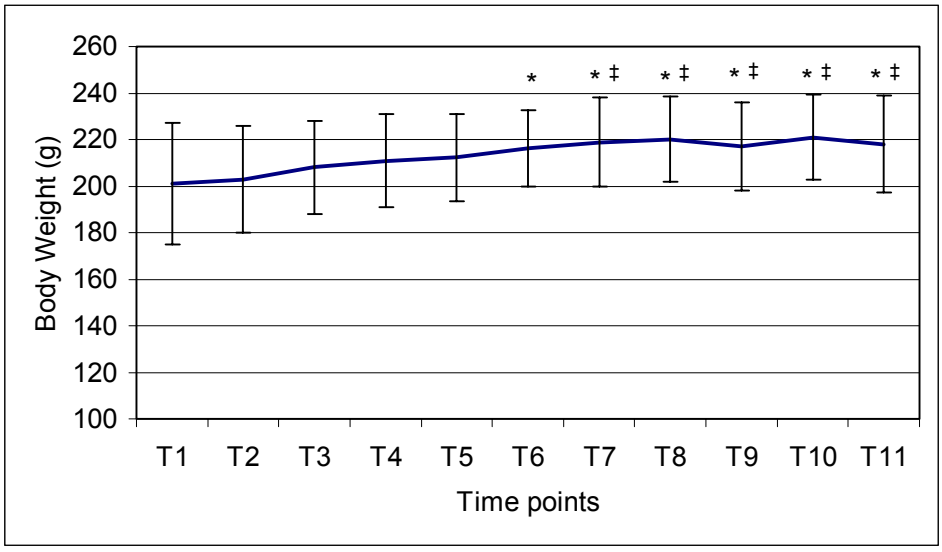


Figure 4.2.2. Body weight across time.
*Denotes significant difference from time point 1
‡ Denotes significant difference from time point 2.
Statistical significance at $p < 0.000$, Student Newman-Keuls significant difference test.

animals had lower weights compared to the Ex and control animals. However, for both wet and dry measures, only the ExHSU group had a significantly lower weight than the Ex group. Figures 4.3.1 and 4.3.2 depict soleus wet and dry weights for each group, respectively.

Soleus mean muscle-weight to body-weight ratios ($g \cdot kg^{-1}$ BW) did not differ between groups (p = 0.921). EDL mean muscle-weight to body-weight ratios ($g \cdot kg^{-1}$ BW) were significantly different (p = 0.042) between groups as depicted in Figure 4.3.3. The ExHSU group had greater muscle-weight to body-weight ratios compared to the control group. Muscle-weight to body-weight ratios can be viewed in Table 4.3.1.

Table 4.3.1. Mean muscle weights and muscle-weight to body-weight ratios.

VARIABLE	GROUP				
	ExHSU	HSU	Ex	Control	p =
Soleus Wet Weights (mg)	92.1 ± 11.4*	95.2 ± 14.6	108.8 ± 12.6*	101.2 ± 15.3	0.028
Soleus Dry Weights (mg)	64.3 ± 13.7*	72.5 ± 15.4	85.0 ± 18.1*	80.5 ± 17.6	0.036
Soleus muscle-weight to body weight ratio ($g \cdot kg^{-1}$ BW)	.4567 ± .0457	.4568 ± .0678	.4790 ± .0436	.4601 ± .0440	0.921
EDL Wet Weights (mg)	92.4 ± 13.4	87.8 ± 6.9	100.3 ± 13.3	91.1 ± 17.0	0.168
EDL Dry Weights (mg)	63.5 ± 12.7	57.7 ± 16.0	73.3 ± 11.1	63.7 ±17.9	0.110
EDL mean muscle-weight to body-weight ratio ($g \cdot kg^{-1}$ BW)	.4578 ± 0552*	.4169 ± .0487	.4339 ± .0386	.4061 ± .0386*	0.042

Values are means ± SD.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise, EDL, extensor digitorum longus.
*Denotes difference from each other, Student Newman-Keuls significant difference test.

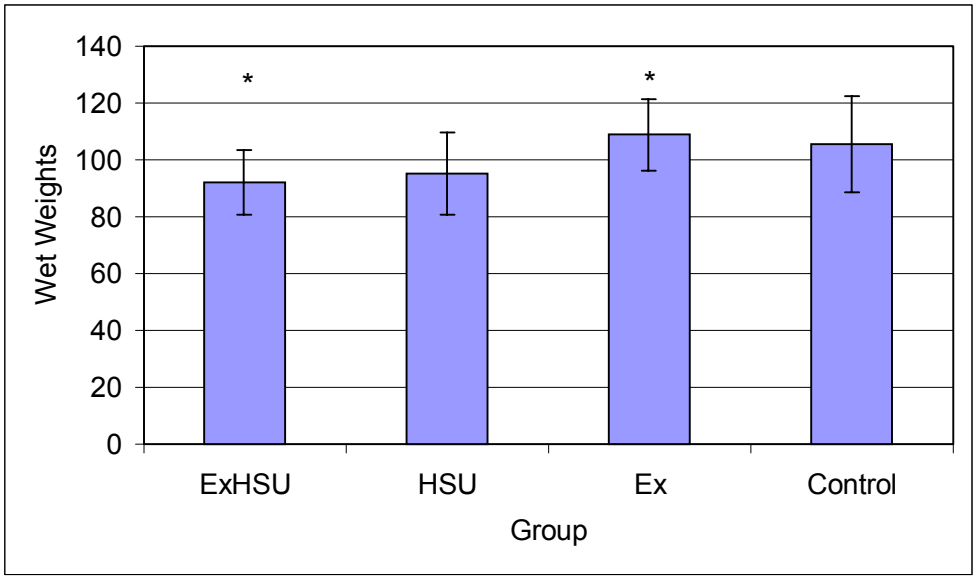


Figure 4.3.1. Soleus wet weight.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes a significant difference between the two groups at $p = 0.028$, Student Newman-Keuls significant difference test

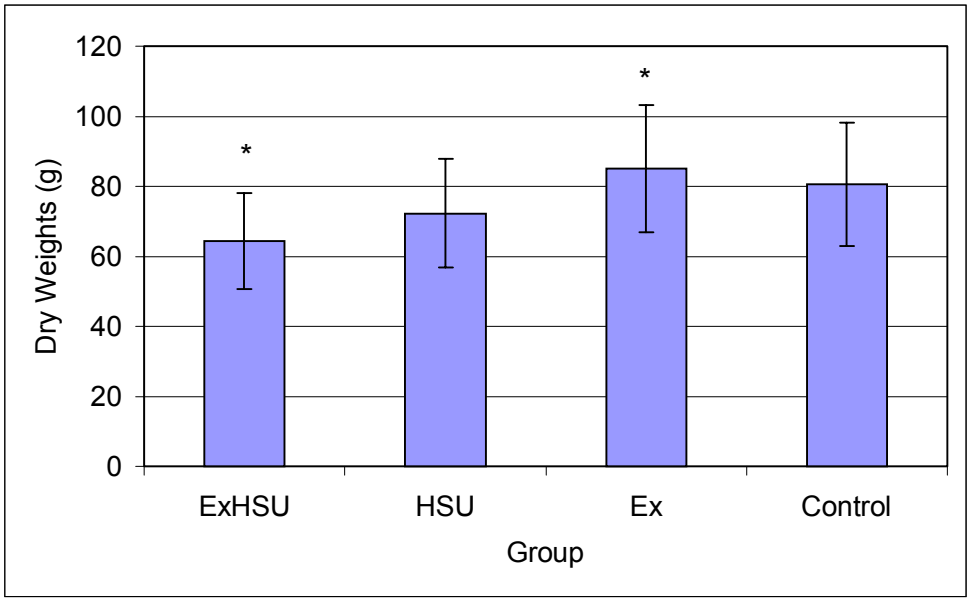


Figure 4.3.2. Soleus dry weight.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb Suspension unloading; Ex, exercise.
*Denotes a significant difference between the two groups at $p = 0.036$, Student Newman-Keuls significant difference test.

4.4 Enzyme Activity

Table 4.4.1 depicts mean glucose-6-phosphate dehydrogenase (G-6-PDH) activity for each group for the right SOL and EDL. There was a significant (p = 0.004) group difference in G-6-PDH activity for the soleus. G-6-PDH activity in the HSU group was +34.8% and +41.6% significantly higher than the Ex and control animals, respectively (Figure 4.4.1). G-6-PDH activity in the ExHSU group, while higher than both the Ex and control groups, did not attain statistical significance. Further, G-6-PDH activity between the ExHSU and HSU groups approached a significant difference (p = .134). Mean G-6-PDH activity for the EDL did not differ between groups.

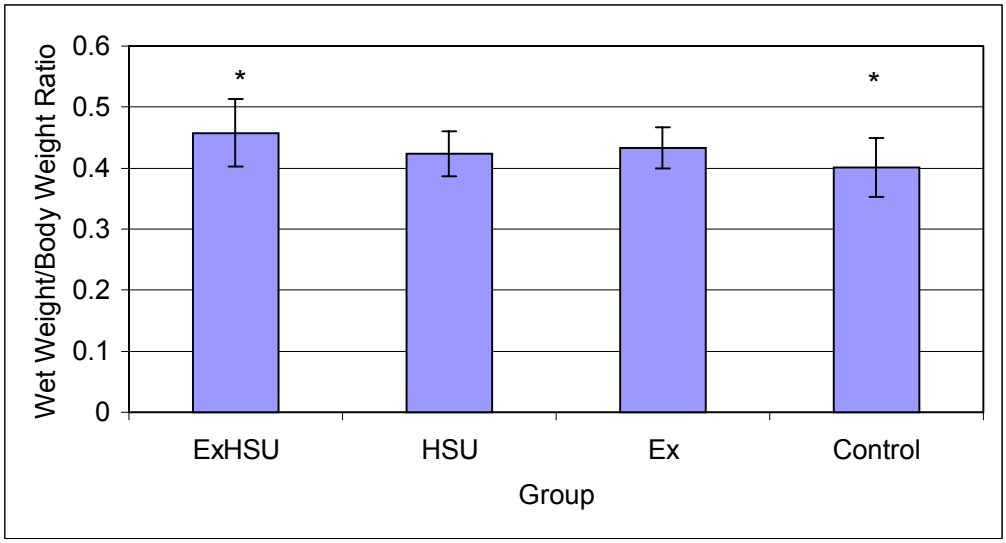


Figure 4.3.3. Extensor digitorum longus muscle-weight to body-weight ratio.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes significant difference between the two groups at p = 0.042, Student Newman-Keuls significant difference test.

Table 4.4.1. Glucose-6-phosphate dehydrogenase activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$).

	GROUP				
	ExHSU	HSU	Ex	Control	*p* =
Soleus	3.48 ± .91	4.23 ± 1.60	2.73 ± 1.09 *	2.47 ± .81*	0.004
EDL	1.85 ± .59	1.38 ± .77	1.62 ± .58	1.11 ± .74	0.193

Values are means ± SD
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise; EDL, extensor digitorum longus.
*Denotes difference from the HSU group at *p* = 0.004, Student Newman-Keuls significant difference test.

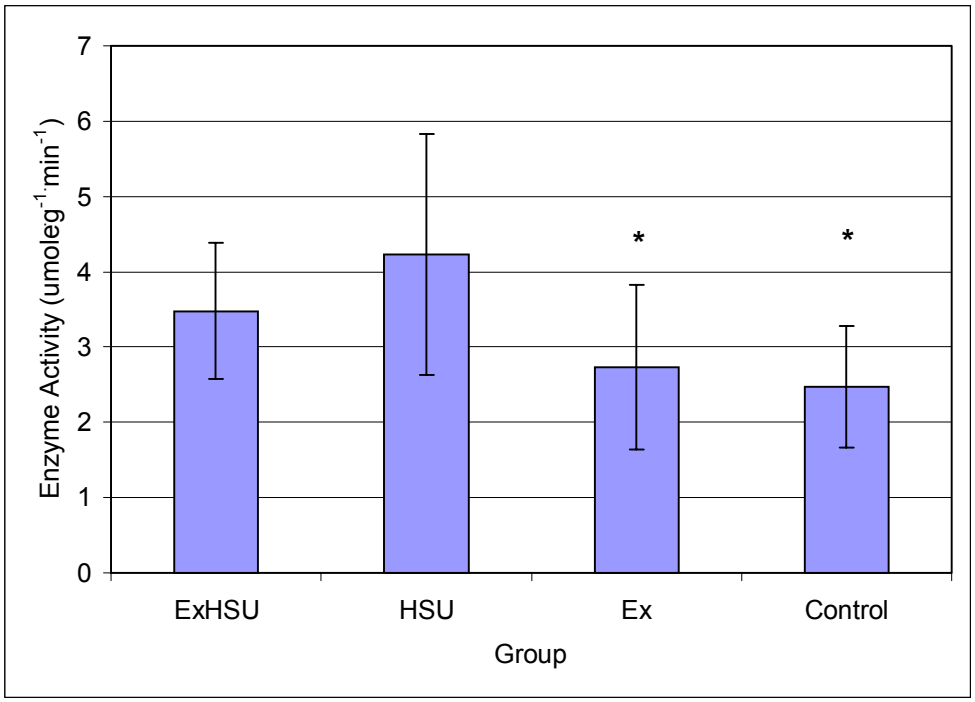


Figure 4.4.1. Soleus glucose-6-phosphate dehydrogenase activity.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading, Ex, exercise.
*Denotes significant difference from HSU group at *p* = 0.004, Student Newman-Keuls significant difference test.

4.5 Fiber Area

Mean fiber areas and fiber area to body-weight ratios for each group are presented in Table 4.5.1. Mean fiber areas for the ExHSU and HSU groups were

significantly smaller than both the Ex and control groups. However, even though the ExHSU group had a smaller mean fiber area than the HSU group, there was no significant difference between the two. Fiber areas and fiber areas to body-weight ratios are depicted in Figure 4.5.1 and Figure 4.5.2, respectively. Fiber area to body-weight ratio indicate that the soleus muscles of the ExHSU and HSU groups experienced muscle atrophy (p = 0.086) during the experimentation (Figure 4.5.2).

Table 4.5.1. Fiber area and fiber area to body-weight ratio for the soleus.

	GROUP				
Fiber Area (μm^2)	ExHSU	HSU	Ex	Control	P =
	2077.7 ± 827.1	2093.6 ± 595.4	3061.1 ± 522.3 *	2827.7± 760.8 *	0.002
Fiber Area to Body-weight Ratio ($cm^2 \cdot kg^{-1}$)	1.03 ± .40	1.01 ± .31	1.32 ± .22†	1.28 ± .42†	0.086

Values are means ± SD.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
* and † denotes significant difference from the ExHSU and HSU groups at p = 0.002 and p = 0.086, respectively, Student Newman-Keuls significant difference test.

4.6 Fiber Damage

Histological data from soleus longitudinal sections are presented in Table 4.6.1. There were no significant differences in the amount of muscle fiber area measured (total muscle area measured) between all groups (p = 0.276). A Levene's homogeneity of variance test was conducted on the data for percent myofibrillar damage and determined that the variance was not equal. To protect against a Type 1 error, a Kruskal-Wallis ANOVA on ranks and a Dunn's multiple comparison procedure was

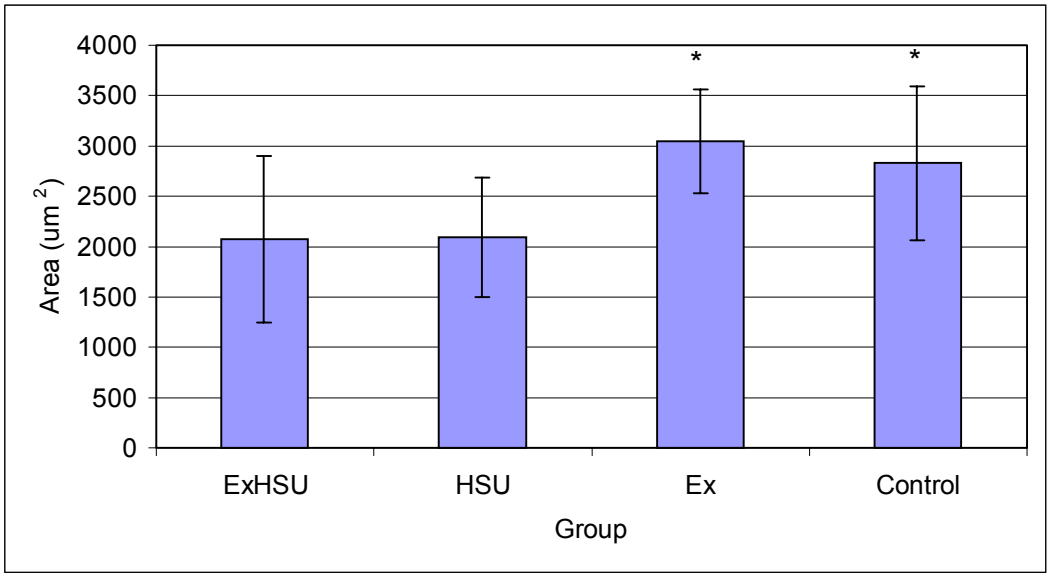


Figure 4.5.1. Mean fiber area for the soleus.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes significant difference from both the ExHSU and HSU groups at p = 0.002, Student Newman-Keuls significant difference test.

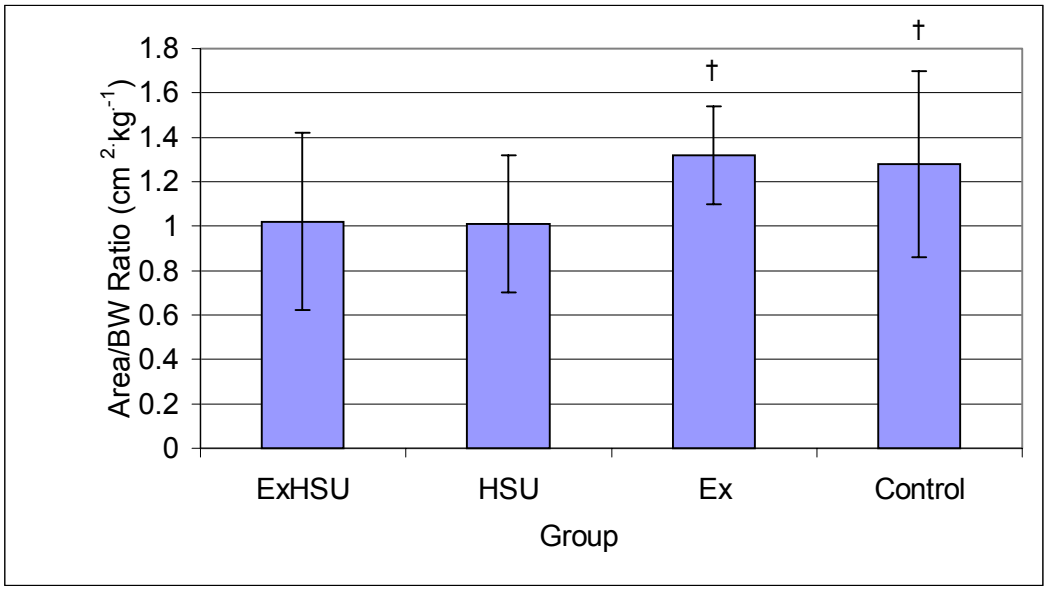


Figure 4.5.2. Fiber area to body weight ratio.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
† Denotes significant difference from both the ExHSU and HSU groups at p = 0.086, Student Newman-Keuls significant difference test.

performed and the results presented in Table 4.6.1 and in Figure 4.6.1. The non-parametric test revealed that the ExHSU and HSU groups are significantly different from both the Ex and control groups. However, the ExHSU group was not significantly different from the HSU group. A post analysis t-test was conducted on percent myofibrillar damage between the ExHSU and HSU groups (Figure 4.6.2) and revealed that the group means indeed were statistically significant (p = 0.065). The comparison of group means in this manner was not in the original statistical design, however, this analysis corresponds with the proposed hypothesis.

Table 4.6.1. Longitudinal fiber damage.

	ExHSU	HSU	Ex	Control	p =
Total Muscle Area Measured (mm^2)	177569.4 ± 9062.4	174737.1 ± 15109.1	185763.0 ± 16124.7	186108.2 ± 20920.0	0.273
Percent myofibrillar Damage	1.49	3.63	0.01*	0.05*	0.001
Percent myofibrillar Damage (t-test)	2.08 ± 1. 54‡	5.03 ± 4.28‡			0.065

Total area measured and percent myofibrillar damage (t-test): Values are means ± SD.
Percent myofibrillar damage: Median value represented.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes significant difference from the ExHSU and HSU groups at p = 0.001, Dunn's multiple comparison procedure.
‡Denotes significant difference from ExHSU and HSU groups at p = 0.065.

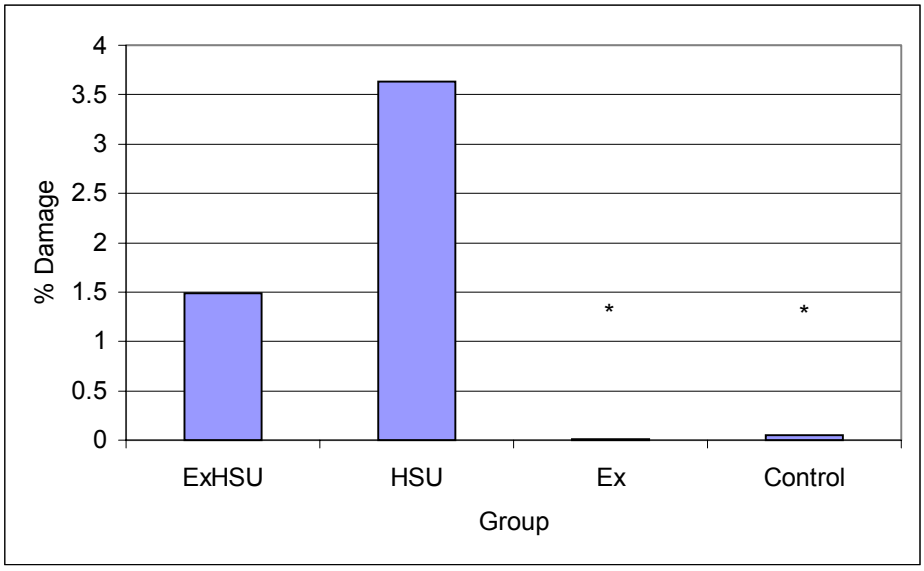


Figure 4.6.1. Myofibrillar fiber damage determined by the Kruskal-Wallis ANOVA on ranks.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
*Denotes significant difference from the ExHSU and HSU groups at $p = 0.001$, Dunn's multiple comparison procedure.

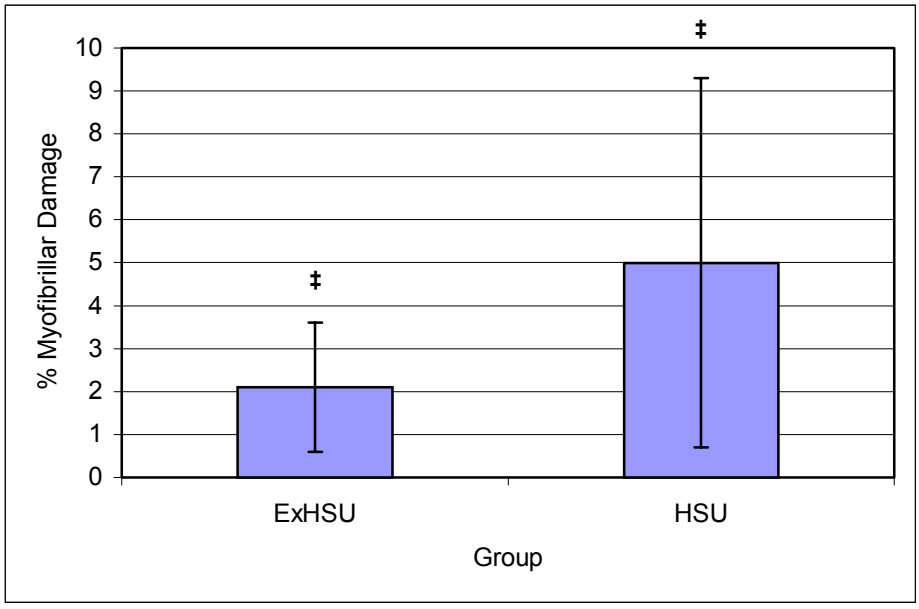


Figure 4.6.2. Comparison of group means (t-test) for myofibrillar damage.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading.
‡Denotes significant difference ($p = 0.065$) between groups.

4.7 Interstitial Area

Table 4.7.1 presents the data calculated for interstitial area. Percent of interstitial area between groups did not significantly differ (p = 0.152). However, as can be seen from the data, the ExHSU and Ex groups had a numerically reduced amount of interstitial space compared to both the HSU and control groups. Further, the HSU group had a numerically larger percent interstitial area compared to the control group, which may be indicative of cellular edema. Figure 4.7.1 graphically illustrates the relative percents of interstitial area and myofibrillar damage measured between groups. As depicted in the figure, the ExHSU group has a statistically lower percent myofibrillar damage (blocked bars) and a numerically lower percent interstitial area (striped bars) when compared to the HSU group. Further, the Ex groups also has a numerically smaller percent myofibrillar damage and numerically smaller percent interstitial area when compared to the control groups. Numerically larger interstitial areas could be indicative of cellular edema. Notice that the HSU group has a numerically larger percent interstitial area compared to the control group. Even though some of these variables did not attain statistical significance between each other, this graphical illustration of cellular damage (myofibrillar damage and possible cellular edema) indicates that the eccentric exercise protocol provided some protection against reloading damage.

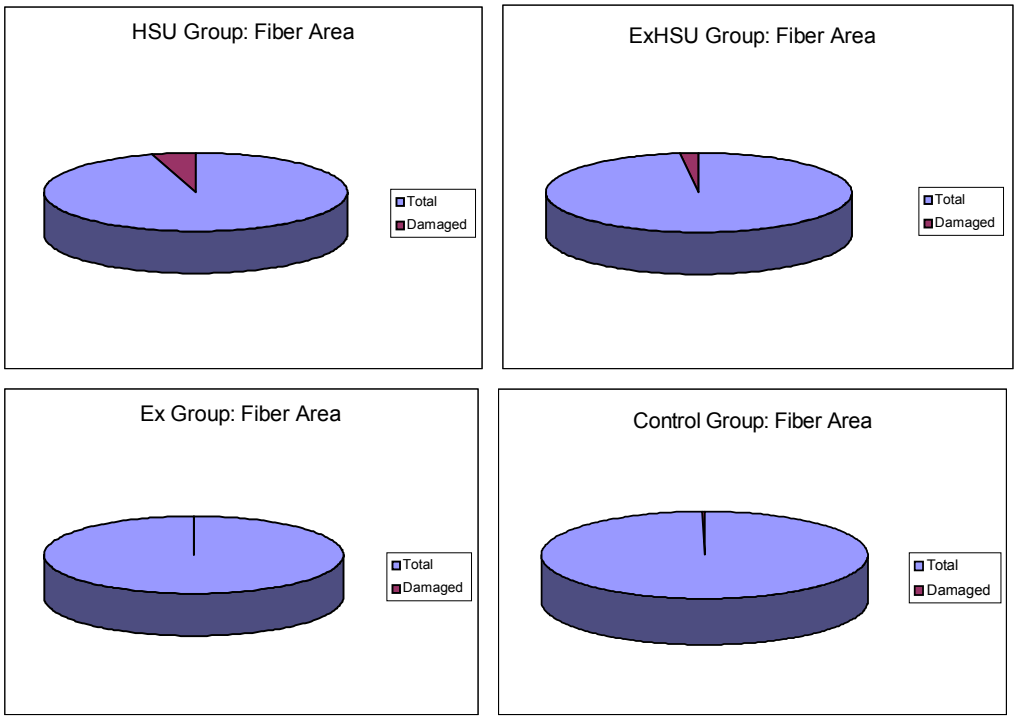


Figure 4.6.3. Graphic representation of percent myofibrillar damage.
a) ExHSU, exercise + hindlimb suspension unloading, b) HSU, hindlimb suspension unloading, c) Ex, exercise only and d) control group. Smaller pies represent damaged area.

Table 4.7.1. Interstitial area.

	ExHSU	HSU	Ex	Control	*p* =
Percent Interstitial Area	8.4 ± 4.7	13.1 ±9.3	7.1 ± 4.9	10.8 ± 5.5	.152

Values are means ± SD.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.

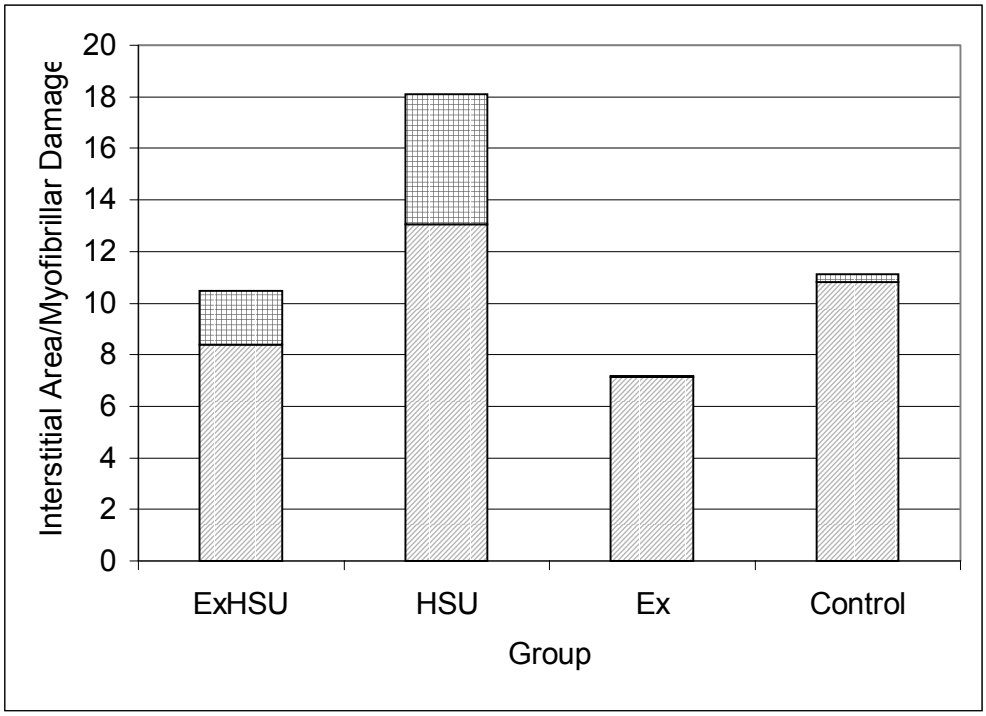


Figure 4.7.1. Relative percents of interstitial area and myofibrillar damage.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.
Lower bars represent percent interstitial area. Lower bars represent percent myofibrillar damage.

4.8 Adrenal Gland Weights, Ratios and Tibia Lengths and Bone Mineral Content

Table 4.8.1 depicts mean adrenal weights, adrenal weight to body-weight ratios and tibia lengths and tibia bone mineral content for each group at the time of sacrifice. There were no significant differences in any of the measures between groups, $p = 0.638$, $p = 0.583$, $p = 0.351$, $p = .651$

4.9 Relationship Between Fiber Area, Myofibrillar Damage, Enzymatic Activity and Interstitial Area

To discern possible relationships between fiber area and the incidence of myofibrillar damage, enzymatic activity and interstitial area, statistical correlations were performed. The analysis revealed a negative correlation ($r = ¯0.158$) between

fiber area and percent myofibrillar damage. The second correlation revealed a positive relationship ($r = {}^{+}0.348$) between percent myofibrillar damage and G-6-PDH activity. The third correlation revealed a positive relationship ($r = {}^{+}0.395$) between percent myofibrillar damage and percent interstitial area. Finally, the fourth correlation revealed a negative relationship ($r = {}^{-}0.462$) between percent interstitial area and fiber area. In other words, the tendency ($p = 0.259$) for smaller fiber areas to become injured during the reloading period was relatively poor. However, G-6-PDH activity increased significantly ($p = 0.072$) and percent interstitial area increased significantly ($p = 0.047$) with the increase in percent myofibrillar damage. Further, percent interstitial area increased significantly ($p = 0.023$) with the decline in fiber area.

Table 4.8.1. Adrenal weights, adrenal-weight to body-weight ratios and tibia lengths and bone mineral content.

	GROUP				
	ExHSU	HSU	Ex	Control	*p* =
Adrenal Weights (mg)	74.1 ± 8.5	73.0 ±12.0	67.8 ± 9.4	68.6 ± 13.6	0.638
Adrenal weight to Body-weight ratio ($g \cdot kg^{-1}$)	.3424 ± .07	.3087 ± .05	.3300 ± .05	.3275 ± .04	0.583
Tibia Lengths (mm)	38.0 ± 1.8	38.6 ± 2.0	37.9 ± 2.5	38.6 ± 1.3	0.351
Tibia Bone Mineral Content	.2225 ± .0374	.2318 ± .0374	.2326 ± .0406	.2448 ± .0361	0.651

Values are means ± SD.
ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise.

Regressions of percent myofibrillar damage on fiber area, G-6-PDH activity, and percent interstitial area were calculated. Likewise, a regression of percent interstitial area on fiber area was calculated. Regression analyses can be viewed

in Table 4.9.1. The regression confirmed the poor relationship between fibers with smaller areas and percent myofibrillar damage. Yet, the higher incidences of myofibrillar damage corresponded to higher G-6-PDH activity and increased interstitial area. Further, fibers with smaller areas at sacrifice tended to have larger percent interstitial area. For example, fiber area only accounts for 3.0% of the variance in myofibrillar damage and was non-significant at p = .259 and F = .376. The coefficient indicates that for each 100 μm^2 increase in fiber area, one would anticipate a 0.14% decline in percent myofibrillar damage (Figure 4.9.1). Yet, myofibrillar damage accounts for 12% of the variance in G-6-PDH activity and is significant at $p < 0.072$ and F = 2.345 and accounts for 15.6% of the variance in percent interstitial area and is significant at p = 0.047 and F = 3.151. Similarly, fiber area accounts for 21% of the variance in percent interstitial area and is significant at p = 0.023 and F = 4.620. The coefficient indicates that for each percent increase in myofibrillar damage, one would anticipate a 0.12 $\mu mole \cdot g^{-1} \cdot min^{-1}$ increase in G-6-PDH activity and a .75% increase in percent interstitial area. Further the coefficient indicates that for each 100 μm^2 decrease in fiber area, one would anticipate a .51% increase in percent interstitial area (Figure 4.9.2).

4.10. Observed power and estimated effect size (R^2)

Table 4.10.1 reports the observed powers and estimated effect sizes (R^2) for the variables related to myofibrillar damage (i.e., SOL G-6-PDH activity, percent myofibrillar damage, percent interstitial area and fiber area). The observed powers for these variables, except for percent interstitial area, indicate

that group sizes were sufficiently large enough to detect treatment effects, if present. Effects sizes of 28.9% (SOL G-6-PDH activity), 46.7% (percent myofibrillar damage), 12.8% (percent interstitial area) and 31.2% (SOL fiber area) of the total variance can be explained by the treatment effect.

Table 4.9.1. Regressions of percent myofibrillar damage on fiber area, G-6-PDH activity, and interstitial area and fiber area on interstitial area.

	UNSTANDARD COEFFICIENT	SE	r^2	F
IV: Fiber Area (μm^2) DV: Myofibrillar Damage (%)	$^{-}$.135	.030	.022	0.376
IV: Myofibrillar Damage (%) DV: G-6-PDH activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)	$^{+}$.116	.076	.121	2.345*
IV: Myofibrillar Damage (%) DV: Interstitial Area (%)	$^{+}$.751	.423	.156	3.151**
IV: Fiber Area (μm^2) DV: Interstitial Area (%)	$^{-}$.509	.237	.214	4.620***

SE, standard error, IV, independent variable; DV, dependent variable; G-6-PDH, glucose-6-phosphate dehydrogenase.
*Denotes significance at p = 0.072.
**Denotes significance at p = 0.047.
***Denotes significance at p = 0.023.

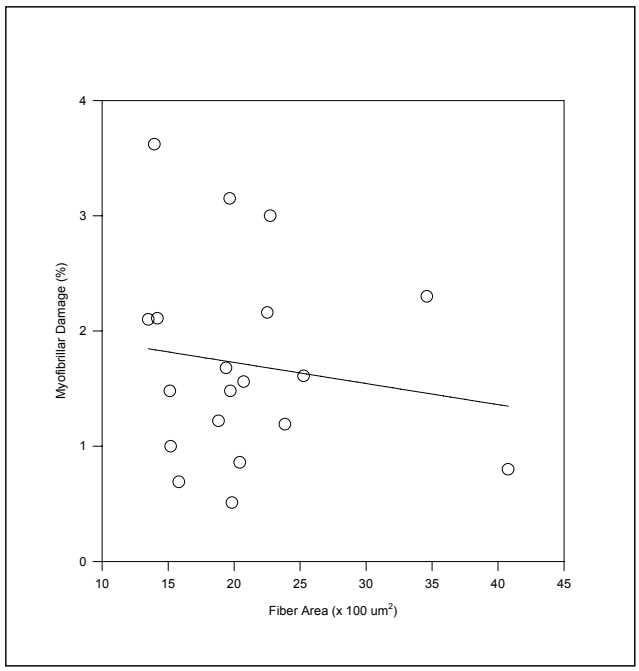


Figure 4.9.1. Regression of percent myofibrillar damage on fiber area for the SOL muscle.

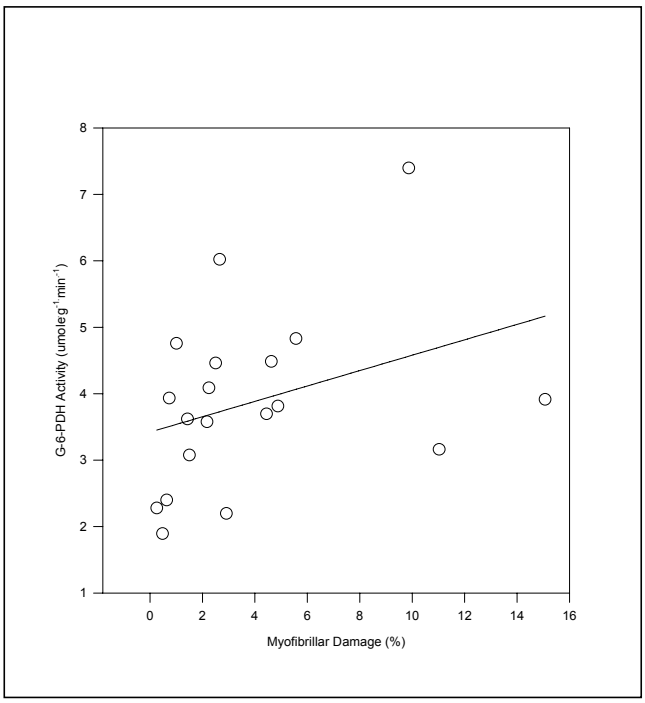


Figure 4.9.2. Regression of percent myofibrillar damage on G-6-PDH activity of the SOL muscle.

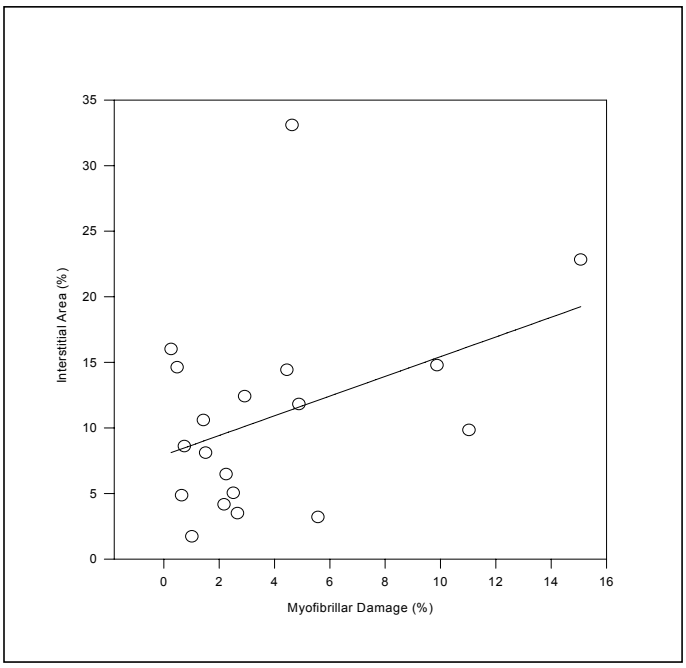


Figure 4.9.3. Regression of percent myofibrillar damage on percent interstitial area of the SOL muscle.

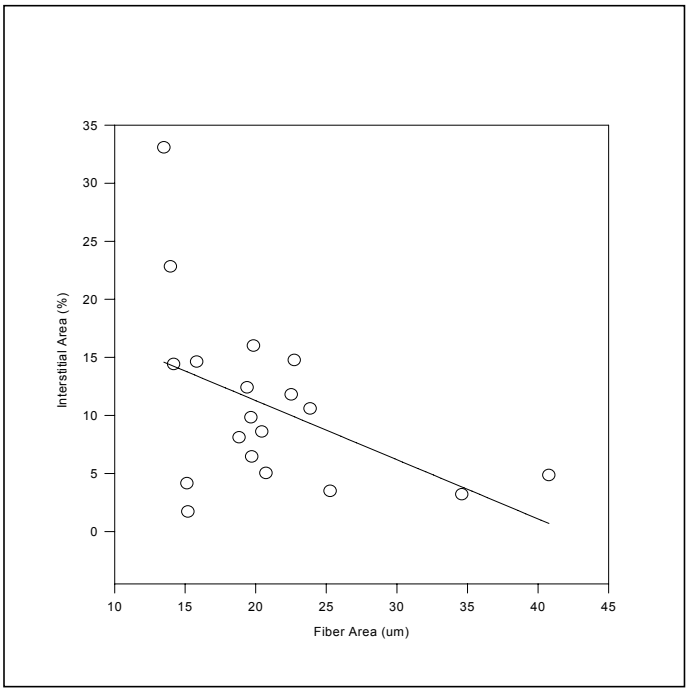


Figure 4.9.4. Regression of percent interstitial area on fiber area for the SOL muscle.

Table 4.10.1. Observed power and estimated effect size (R^2) for G-6-PDH activity, percent myofibrillar damage, percent interstitial area, and fiber area.

Variable	Observed Power	R^2	R^2 Percent
SOL G-6-PDH Activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)	.950	.289	28.9%
Percent Myofibrillar Damage in the SOL Muscle	1.00	.467	46.7%
Percent Interstitial Area in the SOL Muscle	.580	.128	12.8%
SOL Fiber Area (μm^2)	.964	.312	31.2%

SOL, soleus; G-6-PDH, glucose-6-phosphate dehydrogenase. R^2, eta squared (estimated effect size).

CHAPTER 5. DISCUSSION

5.1 Validity of the Hindlimb Suspension Unloading Methodology

Due to the nature of the hindlimb suspension unloading model, it is important to establish the validity of the protocol in order to distinguish between the physiological responses induced by unloading from the stress-related responses induced by physical restraint. For example, normal body weight maintenance is a positive indicator that the animal is tolerating the experimental protocol [144]. The maintenance of normal adrenal gland activity during unloading is another example of tolerance of the experimental protocol. Adrenal gland hyperactivity has been reported during space flight in rodents as an increase in adrenal mass [145] and by a 3-fold increase in urinary corticosteriod levels (1.71 $mg \cdot d^{-1}$ vs. the baseline of 0.5 $mg \cdot d^{-1}$) in rhesus monkeys during the 1^{st} 3 d chair restraint [146]. In these instances, the stressful experimental conditions induced physiological changes within the animal. Stress-related physiological alterations confound the expected experimental outcomes. Therefore, control variables (e.g., EDL G-6-PDH activity, body weight across time, EDL wet and dry muscle weights, adrenal gland weight, etc.) are measured to determine whether the animal is responding to HSU in the anticipated manner. Controls variables should not markedly change during HSU. Therefore, the lack of significant change in the control variables indicates that stress-related physiological alterations did not confound the physiological alterations, if any, produced by the treatment effect.

Morey-Holton, in conjunction with NASA-Ames Research Center, established criteria for an acceptable model of HSU [69]. The criteria state that 1) the model should not stress the animal. Stress is considered minimal if the animals continue to gain body weight similar to controls. 2) Corticosteriod levels in unloaded animals should not differ from controls. 3) The model should not restrict movement of the hind limbs and the animals should be able to recover subsequent to unloading if the experimental design allows for reloading. 4) The pattern of atrophy should be similar to that reported during space flight (i.e., only antigravity muscles lose mass, while other muscle groups demonstrate delayed growth or do not differ from controls) [69].

The results of this investigation provided insight into the overall well being of the animals during the experimentation, especially those groups subjected to suspension. Based upon the morphological and physiological data collected and analyzed in the present investigation (i.e., adrenal weight, body weight, tibia length, muscle atrophy, and the G-6-PDH activity), it was concluded that the HSU protocol did not induce significant stress-related alterations in the animals. Further, this data also supports the criteria established by Morey-Holton and NASA. The results are discussed below.

5.2 Adrenal Weight

In accordance with other investigations [70, 74-76], the suspension period did not increase adrenal weights above those of the control and/or Ex groups. Further when expressed relative to body size, adrenal glands in all animals grew

proportional to their respective body weights. This is especially critical because it suggests that the stress was minimal during the experimentation and not sufficient enough to induce significantly measurable morphological changes within the animal. Adrenal hypertrophy (i.e., higher adrenal weight to body-weight ratios) would indicate that the gland was producing significant amounts of corticosteroids during the suspension period, which would lead to muscle atrophy and complicate interpretation of the results.

5.3 Body Weight

The protocol apparently blunted weight gain in the suspended groups. Body weight at sacrifice was -10.8% (ExHSU) and -8.3% (HSU) lower than the control group. Likewise, when compared to the Ex group, the ExHSU and HSU groups had -12.6% and -10.2% lower body weights, respectively. Several studies have reported declines in overall body mass [9, 19, 75-80, 107, 147] during space flight or simulated conditions. For example, Armstrong et al. (1993) reported an 8.6% loss in body weight during 11 d of HSU in mice. Further, Steffen et al. (1990) reported 5-10% body weight decrements in juvenile Sprague-Dawley rats during 14 d of suspension. The declines in body weight reported by Armstrong et al. (1993) [148] and Steffen et al. (1990) are similar to those presented herein.

Overall body weight was significantly higher at sacrifice (220.9 g) than when the investigation commenced (201.1 g), indicating that stress experienced by the suspended groups did not inhibit overall growth. Further, the lack of an interaction (group x time, $p = 0.916$) supports this conclusion. In other words, no group

significantly gained or lost more weight than any other group during the course of the experimentation. During the initial days of suspension, BW declined in both suspension groups (data not presented), but BW increased as the duration of suspension continued.

The initial decline in body weight demonstrated during actual weightlessness or simulated conditions [9, 19, 75, 77-81] may be related to changes in fluid balance. The reduction of hydrostatic pressure (i.e., space flight) or the head down tilt posture (i.e., hindlimb suspension unloading) causes marked shifts of blood and interstitial fluid from the lower extremities to the cranial and thoracic area. The increase in blood volume in the thoracic area results in an increase in venous return, central blood volume, and vascular distention [83]. Subsequent decreases in antidiuretic and antinatriuretic activity culminate in a decreased production of antidiurectic hormone, aldosterone, and renin [83]. The reduced secretion of these hormones (i.e., antidiuretic hormone, aldosterone and renin) causes an increased renal secretion of water, sodium, chlorine and potassium. These hormonal alterations eventually lead to a marked diuresis that occurs within 1-2 d of the commencement of unloading or space flight. Therefore, increased urine output, as opposed to physiological stress, may account for the decline in body weight seen in the suspended animals during the initial days.

5.4 Tibia Lengths and Bone Mineral Content

Tibia lengths for each group were nearly identical at the time of sacrifice (Table 4.6.1), suggesting that the maturation rate of any animal was not altered.

Further, bone mineral content of the tibia was not significantly different between groups. Similar results are presented in another 7 d HSU, where tibia lengths in the control, exercise + suspension and suspension groups were virtually identical [70]. Inhibition of bone growth does not begin until the second week of HSU [140], and therefore the lack of difference in tibia lengths and bone mineral content between groups presented herein is in accordance with other reports.

5.5 EDL Muscle Weight

The lack of significant atrophy in the EDL muscle during suspension also gave validity to the methodology. The EDL wet and dry muscle weights were not significantly different between groups in this experiment. These results are in accord with other investigations that have reported minimal changes in EDL weight following HSU [19, 22, 65]. However, when expressed relative to body weight, the EDL muscles in the ExHSU group had a significantly higher muscle wet-weight to body-weight ratio when compared to the control group. Muscles adapt to alterations in their functional lengths [149]. For example, soleus muscles maintained at lengthened and shortened positions by means of plaster cast adapted by increasing (20%) and decreasing (40%) the number of sarcomeres in series, respectively [149]. Further, increased mass of dorsiflexor muscles has been reported in another HSU investigation, where rabbit tibialis anterior muscle length increased 30% above control during 7 d [32]. Similar to the tibialis anterior, the EDL experiences chronic stretch during HSU because the foot is usually held in a plantar-flexed position. Slow-oxidative EDL muscle fibers were hypertrophied

following the COSMOS 2044 mission. Investigators concluded that chronic lengthening of the EDL during flight stimulated fiber growth [20].

Interestingly, in the present investigation, HSU alone did not cause hypertrophy of the EDL. Yet, the combination of exercise plus suspension caused significant increases in EDL mass relative to body weight. As can be viewed from Table 4.3.1, EDL wet and dry weights for the Ex group were not significantly higher than the other groups. Therefore, the EDL hypertrophy evident in this investigation is attributed to the combination of prior eccentric exercise and the "foot-drop" plantar flexion posture [22] assumed during HSU.

5.6 G-6-PDH Activity of the EDL Muscle

The enzymatic data also gives credence to the suspension methodology utilized in this investigation. G-6-PDH activity in the EDL muscle was not significantly different between groups, indicating that no significant muscle damage in the EDL occurred as a result of reloading following HSU. This was anticipated since the EDL did not atrophy to any significant degree in either suspension groups and was not expected to incur any reloading damage. Since EDL muscles show little or no change during suspension [64], they are often analyzed in comparison to SOL muscles. The increased activity of G-6-PDH in the SOL compared to the lack of change in the EDL further supports the contention that the soleus is active during weight bearing, whereas the EDL is not as heavily recruited.

In conclusion, the continual gain in body weight and bone growth, along with the lack of muscle atrophy (EDL), enzymatic activity (EDL) and adrenal gland hypertrophy gives validity to the unloading methodology utilized in this investigation. Further, these results are similar to those presented by other investigators [19, 32, 65, 69, 70, 74, 75, 140]. Therefore, any physiological and/or morphological alterations can be attributed to unloading and reloading as opposed to the stress induced by the physical restraint (i.e., the HSU methodology).

5.7 Overall Conclusions of the Investigation

The purpose of the present investigation was to test the hypothesis that high-load eccentric exercise training prior to HSU would attenuate reloading myofibrillar damage. In other words, would prior eccentric exercise display a repeated bout effect and reduce the magnitude of skeletal muscle damage following HSU. The initial analysis of the data indicates that the high-load eccentric exercise training was ineffective in significantly reducing the amount of reloading myofibrillar damage. However, a post-analysis t-test revealed a significant difference in percent myofibrillar damage calculated between the ExHSU and HSU groups and suggests that the reloading repeated bout effect hypothesis is worth closer inspection. Further, the direction of the data for percent interstitial area and G-6-PDH activity suggests that following-up investigations pursuing this line of inquiry should not be discounted. As hypothesized, the EDL did not undergo reloading damage as a result of unloading atrophy. The rationales for the

conclusions drawn and possible limitations of this investigation are discussed below.

Following 7 d of HSU and 16-19 h period of reloading, the solei in this investigation had evidence of morphological damage when viewed with a light microscope. Such damage consists of pale patches of widening cross striations in the banding pattern [22, 24] and increased interstitial area in muscle cross-section (Appendix I). The myofibrillar damage was similar in morphology to lesions produced by eccentric exercise in rats [37, 47], mice [95] and in human subjects [43]. Further, damage observed in this study was similar to the eccentric contraction-like sarcomere lesions evident in reloaded atrophic muscles of other investigations [22, 29, 48]. Adductor longus (AL) muscles, following 12.5 d of HSU and 6 h of reloading [29], demonstrated sarcomere lesions that were consistent with the findings presented herein. Similar lesions were also evident in atrophied AL muscles biopsied 8-11 h following 14 d of space flight [22, 48]. Similar to the SOL, AL muscles aid in posture and locomotion and contain a preponderance of slow twitch fibers [29].

The skeletal muscle damage reported in this investigation can be partially attributed to the atrophic condition of the muscle during reloading. A relationship between reloading muscle damage and muscle atrophy has been suggested by other investigators [22, 25, 34]. In other words, muscle fibers with smaller diameters following suspension were associated with higher incidences of reloading muscle damage. However, this investigation reports only a weak

association (r = ¯0.158) between myofibrillar damage and muscle atrophy following 16-19 h of reloading. The association reported herein is markedly smaller than that reported by Kasper et al. (1995). Following 28 d of HSU and a 7 d reloading period, the correlation (r = ¯0.752) between fiber disruption and cell size in SOL muscle was significant ($p \leq 0.05$), accounting for 61% of the variance in disruption [25]. The larger correlation reported by Kasper et al. (1995) subsequent to 7 d of reloading probably includes other factors associated with muscle degradation and the succeeding inflammatory and immune response. Secondary muscle damage peaks at ~3 d after the primary injury and may be related to, among other factors, inflammatory responses and free radical damage [150, 151]. The inflammatory response includes proteolysis by infiltrating macrophages and neutrophils, which causes damage in excess of that initially experienced by the muscle [118]. Therefore, the magnitude of muscle damage 7 d subsequent to the initial injury can be attributed to several factors involved in degeneration and regeneration as well as fiber size (atrophy induced by 28 d of HSU) at the onset of injury.

Despite the poor correlation between fiber area and percent myofibrillar damage (r = ¯.158, p = .259) and a regression analysis that indicates that fiber area only accounts for 3.0% of the variance in percent myofibrillar damage, the estimated effect size in this investigation is moderate. The estimated effect size indicates that 47.6% of the total variance in percent myofibrillar damage can be attributed to the treatment effect (exercise and/or HSU). The percent myofibrillar damage measured in the Ex SOL did not differ from that measured in the control

group (0.04% vs. 0.31%, respectively), therefore, the exercise treatment can be eliminated as a possible contributing factor in the treatment effect.

The myofibrillar damage evident in the SOL muscle gives credence to the significantly smaller fiber areas and fiber areas to body-weight ratios exhibited in the ExHSU and HSU groups. When fiber areas and ratios were analyzed, muscle atrophy was evident in both suspension groups (p = 0.002). The ExHSU group had ¯32.1% and ¯26.5% and the HSU group had a ¯31.6% and ¯26.0% smaller areas than the Ex and control groups, respectively. Further, the ExHSU and HSU groups had significantly (p = 0.086) lower fiber area to body-weight ratios (1.03 $\pm$ 0.40 and 1.01 $\pm$ 0.31, respectively) when compared to the Ex and control groups (1.32 $\pm$ 0.22 and 1.28 $\pm$ 0.42, respectively). Yet, there were no significant differences between the ExHSU and HSU groups for fiber areas and ratios. The percent myofibrillar damage evident in the ExHSU and HSU groups can therefore be partially explained by the atrophy evident in these groups. The myofibrillar damage evident in both suspension groups discount the lack of atrophy demonstrated in ExHSU and HSU SOL muscle weights and wet-weight to body-weight ratios. In other words, SOL muscle weight and wet-weight to body-weight ratios did not differ between the ExHSU and HSU groups and the control group.

The fiber area and fiber area to body-weight ratio data directly contradicts the data for SOL weights and wet-weight to body-weight ratios, which suggests that the solei muscles in all rat grew in proper proportion to their respective body weights. Yet, the gross measurement of muscle weight is not sensitive enough to

detect fiber atrophy. A more direct assessment of muscle atrophy is seen with the measurement of fiber area [8]. Musacchia et al. (1992) presented similar data from a 14 d space flight mission. Vastus medialis muscle weights in the flight animals were significantly smaller than only the vivarium control animals. However, when fiber area was measured, the flight animals had significantly smaller fiber areas than all of the other groups (i.e., the vivarium control, tail-suspended hindlimb unloaded and basal control groups). The assessment of fiber area gives a more accurate estimation of fiber size because it excludes non-muscle tissue (i.e., invading cells, vascular components and connective tissue cells) and interstitial fluid from the measurement. The exclusion of non-muscle tissue in the analysis is especially important when muscles are analyzed subsequent to reloading and the damaged tissue is undergoing an inflammatory response. Inflammatory responses can increase muscle weight and mask fiber atrophy.

The ExHSU group had wet and dry (92.1 $\pm$ 11.4 mg and 64.3 $\pm$ 13.7 mg, respectively) SOL weights and a wet-weight to body weight ratio (.4567 $\pm$ .0457) similar to the control SOL wet and dry (101.2 $\pm$ 15.3 mg and 80.5 $\pm$ 17.6 mg, respectively) weights and wet-weight to body weight ratio (.4601 $\pm$ .0440). Likewise, the HSU group had wet and dry (95.2 $\pm$ 14.6 mg and 72.5 + 15.4 mg, respectively) weights and a wet-weight to body weight ratio (.4568 $\pm$ .0678) similar to the control group. However, SOL wet and dry weights for the ExHSU group were significantly lower (-15.3% and -24.4%, respectively) than wet and dry

weights in the Ex group. The HSU, Ex and control groups had similar SOL weights.

SOL weights between the HSU group and control group did not differ. Yet, the HSU group had significantly lower fiber areas than both the Ex and control groups. The discrepancy between muscle weight and fiber area in the HSU group can be explained by the analysis of percent interstitial area. Even though interstitial area was not significantly different between groups, the HSU group had numerically more interstitial area (4.7%) than the ExHSU group. The smaller numerical measurement in percent interstitial area in the ExHSU group may explain why the ExHSU group had a significantly lower muscle wet weight compared to the Ex group and the muscle wet weight in the HSU group was not significantly different than the Ex group. The numerically greater percentage of interstitial space measured in the HSU group ($^{+}$4.7%) increased the overall muscle weight and possibly eliminated the significance between the Ex group.

The lack of significance between both suspension groups and the control group in SOL muscle weight and wet-weight to body-weight ratio suggests that both suspension groups experienced an inhibition of muscle growth as opposed to a decline in muscle mass (i.e., atrophy). The lack of atrophy evident in the measurement of muscle weight underscores the importance of measuring fiber area in HSU/reloading investigations. The interpretation of muscle weight data without the analysis of fiber area may lead to an inaccurate conclusion regarding

the presence or absence of muscle atrophy, since the presence of interstitial edema may increase the percent interstitial area and increase muscle weight.

Unexpectedly, percent interstitial area measured between groups only approached significance (p = 0.152). Even though the HSU group had an overall higher numerical measurement of percent interstitial area (13.1 $\pm$ 9.3%), it was not significantly elevated above the ExHSU, Ex and control groups (8.4 $\pm$ 4.7%, 7.1 $\pm$ 4.9% and 10.8 $\pm$ 5.5%, respectively). However, the lack of a group difference in percent interstitial area corresponds to SOL muscle data reported after the SLS-1 space flight [8]. The percent interstitial area in these SOL muscles did not increase significantly during any reloading time (2.3-3.3, 4.5-5.5, and 5.8-6.8 h). However, the post-flight times in this investigation are substantially decreased compared to the reloading times of 16-19 h in the current investigation.

Further, adductor longus (AL) interstitial area in SLS-1 and SLS-2 missions did significantly increase following flight [8]. AL interstitial area in the SLS-1 mission increased significantly at 2.3-3.3 hr following flight and continued to increase at the other post-flight times. AL interstitial area in the SLS-2 mission increased significantly at 5 h following flight and remained elevated at 9 d post-flight. Therefore, percent interstitial area in the current investigation should have increased significantly following 16-19 h of reloading, when inflammatory responses should have been in progress.

Interestingly in another investigation, the interstitial areas of AL muscles subjected to space flight were twice as high as the AL control muscles (15% vs.

6.5%, respectively) [22]. However, the control AL muscles had similar interstitial areas when compared to the atrophic AL muscles of the HSU group (6.5% and 7.0%, respectively). The flight animals were subjected to reentry vibration and impact landing as opposed to the suspended animals that were simply let down. The flight animals were sacrificed between 8-11 h post flight and the HSU rats were sacrificed 15-60 min after reloading. The investigators speculated that the increased use of the flight AL muscle during the impact of landing and during the extended reloading time (8-11 h vs. 15-60 min) contributed to the increased interstitial area observed in the AL flight muscles. Also, the phagocytic phase (i.e., inflammatory response) of degeneration occurs 2 to 6 h after the initial injury [71]. The phagocytic phase is responsible for removing injured tissue and initiating regeneration within the damaged fiber. Hence, the interstitial area measured at 15-60 min could not be considered inflamed, since the inflammatory response would not have been initiated. The discrepancy of sacrifice times resulted in the differing measures of interstitial area between the AL flight and HSU muscles.

The percent interstitial area measured in damaged tissue is also dependent upon the amount of voluntary muscle movement during the reloading period. The sacrifice time of 8-11 h in the flight AL muscles [22] is comparable to the sacrifice time of 16-19 h in current investigation. Even though SOL muscles were examined in the current experiment, the interstitial areas are similar to the flight AL muscles. The AL flight muscles had 15% interstitial area compared to 13.1% for the HSU SOL. However, a limitation of this investigation is the lack of control for voluntary

movement of the animals during reloading. Therefore, non-significant differences between groups in percent interstitial area may reflect individual differences in ambulation among the rats. Further, since the magnitude of muscle damage is associated with the amount of voluntary movement and motor unit recruitment during reloading [24], differences in ambulation may also be responsible for the high variation observed in myofibrillar damage for the HSU group.

Myofibrillar damage in the HSU group ranged from .26% - 13.10%. The marked variation in myofibrillar damage measured between the rats in this investigation is similar to high variations reported in human subjects and rats after space flight [45, 152] and in rats following HSU investigations [8, 22, 28, 48, 153]. Following 3 wk of HSU, rat soleus muscle degeneration ranged from 4.66% - 14.08% [154]. Further, considerable variation in cell atrophy and loss of peak force was measured in astronauts following 17 d of space flight [152]. Activity levels of the HSU and ExHSU groups during the reloading period in this investigation are unknown. Assuming the voluntary activity was similar between the two groups, could a repeated bout effect be responsible for the smaller variation in myofibrillar damage (.47%- 4.46%) observed in the ExHSU group when compared to the HSU group? At present, this question cannot be answered.

The Kruskal-Wallis ANOVA on ranks analysis for percent myofibrillar damage revealed significant differences (p = 0.001) between the ExHSU and HSU groups vs. the Ex and control groups. Yet, the ExHSU and HSU groups did not differ significantly (1.49% and 3.63%, respectively). However, a post-analysis

comparison of means (independent t-test) between these two groups revealed a significant difference (p = 0.065) between the ExHSU and HSU groups. In other words, the HSU group had a significantly higher percent myofibrillar damage compared to the ExHSU group (5.03 $\pm$ 4.28 vs. 2.08 $\pm$ 1.54%, respectively). The comparison of group means via an independent t-test was not in the original statistical design. However, the comparison of group means in this manner addresses the proposed question of whether eccentric exercise prior to HSU attenuates reloading damage and specifically compares those groups (ExHSU and HSU) demonstrating muscle damage. Therefore, even though significance between the ExHSU and HSU groups was not demonstrated with the ANOVA, the results of the independent t-test illustrating a significant difference between these groups should not be ignored. Therefore, in regards to percent myofibrillar damage, prior eccentric exercise was effective in attenuating reloading damage and the prior eccentric exercise protocol demonstrated a repeated bout effect.

Figure 4.7.1 may further support the reloading repeated bout effect theory. This figure depicts the relative percents of interstitial area and myofibrillar damage measured for each group. Interstitial area did not differ between groups. Yet, the smaller numerical measurements in percent interstitial area in the ExHSU group vs. the HSU group and the Ex group vs. the control group may indicate less inflammation in muscle cells of the exercised animals. A blunted inflammatory response [59] has been suggested as a possible mechanism of the repeated bout effect. Subsequent to the repeat bout of exercise, neutrophil and monocyte

activation was decreased, resulting in an attenuated inflammatory response [59]. Yet, it is uncertain whether these effects relate to a blunted immune response or the attenuation of tissue damage following the repeat bout [89]. An adaptation in the inflammatory response may have contributed to the numerically lower measurements of interstitial areas in the exercised groups (ExHSU and Ex). However, the ExHSU group had a significantly lower percent myofibrillar damage compared to the HSU group, which may have reduced the magnitude of the succeeding inflammatory response. A limitation to this theory is the lack of significance in percent interstitial area.

The percent interstitial area measured was positively and significantly correlated ($r = {}^{+}0.395$, $p = 0.047$) with percent myofibrillar damage in the ExHSU and HSU groups. Hence, as the percent of myofibrillar damage increased in the muscle cell, the percent of interstitial area increased. Myofibrillar damage accounts for 15.6% of the variance in percent interstitial area and for every percent increase in myofibrillar damage, one can expect a 0.75% increase in interstitial area. Further, percent interstitial area was negatively and significantly ($r = {}^{-}0.462$, $p = 0.023$) correlated with fiber area. In other words, smaller fiber areas were associated with larger percentages of interstitial area in the muscle subsequent to the reloading period. Fiber area accounts for 21% of the variance in percent interstitial area and for every 100 μm^2 decrease in fiber area, one would anticipate a .51% increase interstitial area. The decline in fiber area with a subsequent increase in percent interstitial area corresponds well with the positive and

significant ($r = {}^{+} 0.348$, $p = 0.072$) correlation between percent myofibrillar damage and G-6-PDH activity. Higher percentages of myofibrillar damage corresponded to higher G-6-PDH activity. As hypothesized in this document, the decline in fiber area during HSU is partially responsible for increased myofibrillar damage during reloading. As anticipated, increased myofibrillar damage would activate the pentose phosphate pathway; causing up-regulation in the activity of the rate-limiting enzyme of this pathway: G-6-PDH. As a result of muscle damage and subsequent degenerative and regenerative responses in the cell, increases in percent interstitial area (i.e., edema) may result.

Percent myofibrillar damage accounts for 12% of the variance in G-6-PDH activity and for each percent increase in myofibrillar damage; one would anticipate a 0.12 $\mu mole \cdot g^{-1} \cdot min^{-1}$ increase in G-6-PDH activity. Yet, the low coefficient of determination suggests that other factors are involved in the release of G-6-PDH or that other possible degradative pathways are activated following muscle injury (i.e., loss of intracellular Ca^{++} homeostasis, loss of energy supply to the cell and increased activity of oxidizing free-radical mediated reactions) [155]. Wagner et al (1997) reported a biphasic increase in G-6-PDH activity following Marcaine administration. Marcaine (bupivacaine) is a myotoxic anesthetic that induces acute muscle fiber degeneration followed by complete and rapid regeneration [156]. A comparison in the time course of G-6-PDH activity with β-glucuronidase (i.e., an enzyme enriched in phagocytic cells) revealed that the two activities did not increase in parallel. Therefore, the enhanced G-6-PDH activity present in muscle

fibers during the first 6-8 h after Marcaine administration can be localized within muscle cells [157]. The secondary increase in G-6-PDH activity (15-24 h) corresponded with an increase in β-glucuronidase activity, suggesting that the infiltration of phagocytic cells (including the influx of β-glucuronidase) into the muscle cells contributed to the increased activity of G-6-PDH.

The percent interstitial area in this investigation only approached significance, suggesting that edema was not yet present in the hindlimb suspended unloaded muscles at sacrifice. In other words, there was a delay in the phagocytic phase or it had yet to reach its peak. In either situation, the measurement of G-6-PDH activity during 16-19 h of reloading may have come prior to the secondary increase in this enzymes activity. Investigators have linked this enzyme to proliferation of cells involved in degenerative-regenerative processes [37]. In light of the data presented in this investigation, it appears that G-6-PDH activity is more closely associated with regeneration, since myofibrillar damage is clearly evident in the muscle cell. The measurement of G-6-PDH activity during the influx of phagocytic cells may have revealed an even more marked increased activity of this enzyme.

G-6-PDH activity in the ExHSU group was increased +21.6% and +29.0% above the Ex and control groups, respectively. Further, G-6-PDH activity in the HSU group was increased +35.5% and +41.6% above the Ex and control groups respectively. Yet, even though the ExHSU group had –17.7% lower enzymatic activity compared with the HSU group, this difference only approached

significance (p = .134). Muscular activity of this enzyme has increased after Marcaine administration [157] and in exercise-related investigations [37, 51]. However, this investigation, along with pilot data, is the first to report increased activity of this enzyme subsequent to reloading damage. Since the difference in G-6-PDH activity between the ExHSU and HSU groups approached significance (p = .134), we can appropriately conclude that G-6-PDH activity during the reloading period had a tendency to be lower in groups subjected to the eccentric exercise protocol.

The eccentric exercise protocol partially protected the SOL muscle from reloading damage. The exact mechanism(s) of reloading fiber damage are not known but may in part relate to muscle atrophy and the selective loss of actin myofilaments during unloading [8, 34]. The correlation between percent myofibrillar damage and fiber area was poor in this investigation, suggesting that other factors are involved in muscle damage during reloading. Riley et al. (1996) reported a selective loss of actin myofilaments compared to myosin myofilaments following 17 d of space flight in humans. This alteration presumably increased the spacing between the thick and thin filaments and resulted in a 26% decline in thin filament density in the overlap A-band region [8]. Coupled with muscle atrophy, the alteration in filament distribution resulted in weaker contractile units and can perhaps account for a portion of the variance in muscle disruption.

In the current investigation, 3% of variance in percent myofibrillar damage is accounted for by fiber area. In other words, attenuating the decline in fiber area by

only 100 μm^2 could theoretically reduce percent myofibrillar damage by 0.14% per fiber. The attenuation of muscle atrophy by 100 μm^2 in even a fraction of the thousands of fibers in any given muscle could significantly attenuate reloading damage. Therefore, while the association between myofibrillar damage and fiber area in this investigation is not strong, the potential contribution of muscle atrophy to reloading damage should not be discounted.

Further, the eccentric exercise protocol prior to HSU did not attenuate unloading atrophy (ExHSU; 2077.7 $\pm$ 827.1 μm^2 vs. HSU; 2093.6 $\pm$ 595.4 μm^2). Since body weight in all groups increased during this experiment, the smaller fiber areas in the ExHSU and HSU groups were a result of muscle wasting (i.e., an alteration in the ratio of protein synthesis to protein degradation) as opposed to a retardation of overall growth. Protein turnover was not measured in this investigation. However, declines in protein synthesis are most prominent during the first wk of HSU, whereas degradation peaks between 9-15 d [158]. Therefore, a decline in protein synthesis may be responsible for the muscle atrophy reported herein.

Since the ExHSU and HSU groups experienced similar magnitudes of atrophy, one would anticipate similar magnitudes of myofibrillar damage between these groups. However, percent myofibrillar damage in the ExHSU group was significantly lower than in the HSU group (2.08 $\pm$ 1.54% vs. 5.03 $\pm$ 4.28%, respectively), suggesting mechanisms, other than the attenuation of muscle atrophy, may be involved in the reduction of reloading myofibrillar damage. Hence,

perhaps the eccentric exercise training protocol elicited physiological adaptations within the SOL muscle that evoked a repeated bout effect during muscular reloading.

The cellular theory of the repeated bout effect proposes that new proteins (e.g., cytoskeletal proteins) are synthesized as a result of exercise-induced damage. These newly synthesized proteins are more resilient to damage induced by subsequent bouts of exercise. Lengthening contractions cause injury to the muscle in two stages: 1) a primary insult that is mechanical in nature and 2) secondary damage that peaks ~3 d after the exercise bout [95]. The initial injury is perhaps the result of stronger sarcomeres pulling weaker sarcomeres apart [96, 159]. A potential mechanism of the repeated bout effect may be the strengthening of the cytoskeletal protein network that surrounds the sarcomeres [95]. The synthesis of proteins in this network (i.e., desmin, talin, vinculin, dystrophin) may stabilize sarcomeres and protect the muscle from future injuries [95]. Increased synthesis of such damage-resistant proteins as a result of the eccentric exercise training protocol may have attenuated the magnitude of damage during reloading by strengthening the sarcomere and consequently may have provided a repeated bout effect. Therefore, despite the muscle atrophy that occurred in both suspension groups, the eccentric exercise training protocol might have strengthened the cytoskeletal network in the sarcomeres of the ExHSU group, resulting a lower percent of myofibrillar damage during the reloading period.

Therefore, prior muscle conditioning (i.e., high-load eccentric exercise) may be an effective countermeasure in the protection against reloading damage, even though it did not protect against unloading atrophy.

The benefits of eccentric exercise training on reloading myofibrillar damage subsequent to HSU presented in this investigation contradict a similar phenomenon reported in the cardiovascular system. In the cardiovascular system, orthostatic intolerance following unloading and space flight is more exacerbated in highly trained individuals as opposed to individuals who are less conditioned [126]. In other words, higher maximal oxygen consumptions ($VO_{2\ max}$) prior to unloading or space flight results in a greater decline in $VO_{2\ max}$ subsequent to unloading or space flight. Generally speaking, in the musculoskeletal system, the greatest magnitude of atrophy occurs in fibers that are the largest prior to suspension or space flight [84, 112, 152]. For example, soleus type I fibers have significantly larger diameters than gastrocnemius type I fibers. The difference in diameter size may account for the greater magnitude of atrophy often reported in soleus type I fibers compared to gastrocnemius type I fibers [34]. Yet, increasing fiber area prior to HSU via an exercise training protocol may not demonstrate the same magnitudes of muscle atrophy during the HSU period.

In the current investigation, the Ex SOL fibers were not significantly larger than the control SOL fibers. However, fiber area was not measured prior to HSU. Therefore, it is unknown whether the exercise protocol elicited hypertrophy in the SOL muscle prior to HSU and at present, this question cannot be answered. The

attenuation of myofibrillar damage during reloading was the focus of this investigation. Since eccentric exercise induces myofibrillar damage, the exercise protocol had to be carefully designed to eliminate this possibility. A 10 d period between the cessation of exercise and sacrifice was necessary to avoid complicating the results by inducing damage with the exercise protocol. Yet, the question remains whether hypertrophy elicited by prior exercise training would attenuate unloading atrophy. A follow-up investigation specifically designed to address this experimental limitation would have to be undertaken.

CHAPTER 6. CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusion

1. The high-load eccentric exercise training protocol prior to HSU resulted in: a) Reduced myofibrillar damage in the ExHSU group vs. the HSU group, b) a tendency (p = .152) towards reduced interstitial area in the ExHSU group vs. the HSU group, and c) a tendency (p = .134) for lower G-6-PDH activity in the ExHSU group vs. the HSU group.
2. Further, the eccentric exercise training protocol prior to HSU had no effect on the magnitude of muscle atrophy during the suspension period.
3. As anticipated, the EDL muscle did not demonstrate a differential response in G-6-PDH activity between groups.
5. As expected, the EDL muscle did not exhibit significant muscle atrophy in either suspension group (ExHSU and HSU).
6. Despite the lack of significant differences in percent interstitial area and G-6-PDH activity, the significant difference observed between the ExHSU and HSU groups in percent myofibrillar damage suggests that this particular exercise protocol was effective in evoking a repeated bout effect during reloading.
7. Even though there were no significant differences measured in percent interstitial area and G-6-PDH activity, the direction of the data indicates that further investigations are warranted.

8. This investigation, coupled with the pilot investigation, is the first to report increased G-6-PDH activity subsequent to HSU and reloading.
9. Further, this investigation was the first to assess whether the repeated bout effect was applicable to reloading injury. The conclusions presented herein should be assessed according to this fact and more investigations into this matter undertaken.

Sarcomeres have a physical threshold to damage that is reached with eccentric exercise [94] and decreased with atrophy [29]. Observation of the results indicates that the exercise protocol seemed to partially maintain this physical threshold limit. For example, the numerical measurements of percent interstitial area was lower in the ExHSU group compared to the HSU group and lower in the Ex group compared to the control group. Also, the ExHSU and HSU groups had similar amounts of atrophy, yet the percent of myofibrillar damage calculated was less in the ExHSU group. Graphic illustrations of these points can be viewed in Figure 4.7.1. Further, the comparison between means (ExHSU and HSU) for percent myofibrillar damage indicates that reloading damage was attenuated in the ExHSU group. Despite the fact that the comparison of means in this manner (t-test) was not in the original statistical design, the outcome of this analysis should be considered fully when interpreting the results of this investigation. Therefore, follow-up investigations are appropriate in order to support or discount the conclusions reached in this document.

6.2 Future Directions

Follow-up investigations can take several directions; which include 1) the determination of peak G-6-PDH activity, 2) assessing the relationship between G-6-PDH activity and myofibrillar damage, 3) determination of a more appropriate exercise protocol, 4) assessment of the functional properties of the muscle and 5) assessment of detraining on myofibrillar size.

1. This investigation was the first to demonstrate increased G-6-PDH activity during 16-19 h of reloading. Since the exact time course of G-6-PDH activation and peak activity following reloading damage has yet to be assessed, future investigations should include time course experimental protocols to determine the hours of peak activity for this enzyme during reloading.
2. The relationship between G-6-PDH activity and myofibrillar damage was not highly correlated in this investigation. It would be beneficial to determine when G-6-PDH activity peaks in relation to myofibrillar destruction. Several investigations have implicated G-6-PDH activity in degenerative and regenerative processes. Time course experimental protocols that assess myofibrillar disruption in relation to fluctuations in G-6-PDH activity would clarify the role of this enzyme in both degenerative and regenerative pathways.
3. The exercise protocol in this investigation seemed to partially maintain the physical threshold to damage in the sarcomeres. Yet, significance in some

experimental variables (i.e., percent interstitial area and G-6-PDH activity) was not attained. Therefore, another exercise design may be more effective in eliciting a significant response in these variables. Herbert et al. (1988) utilized various exercise protocols in attempts to attenuate unloading atrophy. One of those protocols served as a template for the exercise protocol utilized in this investigation. Manipulation of the exercise protocol (i.e., increasing the number of repetitions and/or sets performed, and/or increasing the duration of the training period) may elicit significant differences between the ExHSU and HSU groups in those variables that were non-significant in this investigation.

4. The functional properties of the muscle following HSU and reloading needs to be assessed in future investigations. Investigations that have demonstrated repeated bout effects following unaccustomed eccentric exercise have reported decrements in muscle function following the initial bout. Further, muscle properties are altered following hindlimb suspension unloading. With the addition of the exercise component, assessment of the functional alterations elicited by exercise, suspension and reloading would be interesting. Therefore, a possible future direction would include the measurement of muscle function.
5. Finally, the effects of detraining in this experimental protocol should be assessed in future investigations. Since fiber area was not determined until sacrifice, it is not known whether the exercise protocol elicited SOL

hypertrophy. Perhaps the 10 d of detraining between the cessation of exercise and sacrifice caused a decrease in fiber area in the SOL muscle. Time course follow-up investigations should attempt to answer this question.

As stated earlier, the high-load eccentric exercise protocol was effective in attenuating reloading myofibrillar damage and demonstrated a trend in percent interstitial area and G-6-PDH activity. However, the novelty of this investigation requires that follow-up studies be conducted that either disprove or support the hypothesis suggested herein. Manipulation of some or all of the experimental protocols could possibly answer pertinent questions regarding unloading-induced muscle atrophy and reloading damage in association with prior exercise, a possible repeated bout effect, functional properties, and degenerative/regenerative processes.

REFERENCES

1. Clarke, M.S.F., M.M. Bamman, and D.L. Feeback., *Bed rest decreases mechanically induced myofiber wounding consequent wound-mediated FGF release.* J. Appl. Physiol., 1998. 85(2): p. 593-600.

2. Appell, H.J., *Skeletal muscle atrophy during immobilization.* Int. J. Sports Med., 1986. 7: p. 1-5.

3. Jarvinen, M.J., and M.U.K. Lehto., *The effects of early immobilization and immobilization on the healing process following muscle injuries.* Sports Med., 1993. 15: p. 78-89.

4. Larsson, L., G. Grinby, and J. Karlsson., *Muscle strength and speed of movement in relation to age and muscle morphology.* J. Appl. Physiol., 1979. 46: p. 451-456.

5. Lexell, J., C.C. Taylor, and J. Karlsson., *What is the cause of the ageing atrophy?* J. Neurol. Sci., 1988. 84: p. 275-294.

6. Youmans, J.R., and A.H. Smith, *Gravitational Fields and Aging.* The Physiologist, 1991. 34(1): p. S19-S22.

7. LeBlanc, A., R. Rowe, V. Schneider, H. Evans, and T. Hedrick., *Regional muscle loss after short duration spaceflight.* Aviat. Space Environ. Med., 1995. 66: p. 1151-1154.

8. Riley, D.A., S. Ellis, G.R. Slocum, F.R. Sedlak, J.L.W. Bain, B.B. Krippendorf, C.T. Lehman, M.Y. Macias, J.L. Thompson, K. Vijayan and J.A. DeBruin., *In-flight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats.* J. Appl. Physiol., 1996. 81: p. 133-144.

9. Caiozzo, V.J., M.J. Baker, R.E. Herrick, M. Tao and K.M. Baldwin, *Effects of spaceflight on skeletal muscle: mechanical properties of myosin isoform content of a slow muscle.* J. Appl. Physiol., 1994. 76: p. 1764-1773.

10. Deschenes, M.R., A.A. Britt, and W.C. Chandler., *A comparison of the effects of unloading in young adult and aged skeletal muscle.* Med. & Sci. Sports & Exerc., 2001. 33(9): p. 1477-1483.

11. Berg, H.E., G.A. Dudley, T. Haggmark, H. Ohlsen, and P.A. Tesch., *Effects of lower limb unloading on skeletal muscle mass and function in humans.* J. Appl. Physiol., 1991. 70: p. 1882-1885.

12. Dudley, G.A., M.R. Duvoisin, V.A. Convertino and P. Buchanan., *Alterations of the in vivo torque-velocity relationship of human skeletal muscle following 30 days exposure to simulated microgravity.* Aviat. Space Environ. Med., 1989. 60: p. 659-663.

13. LeBlanc, A., P. Gogia, V. Schneider, J. Krebs, E. Schonfeld, and H. Evans., *Calf muscle area and strength changes after five weeks of horizontal bed rest.* Am. J. Sports Med., 1988. 16: p. 624-629.

14. MacDougall, J.D., G.C.B. Elder, D.G. Sale, J.R. Moroz, and J.R. Sutton., *Effects of strength training and immobilization on human muscle fibers.* Eur. J. Appl. Physiol., 1980. 43: p. 25-34.

15. Council, S.S.B.N.R., *A Strategy for Research in Space Biology and Medicine in the New Century*. 1998, Washington, D.C.: National Academy Press. 97-117.

16. Riley, D.A., J.L.W. Bain, J.L. Thompson, R.H. Fitts, J.J. Widrick, S.W. Trappe, T.A Trappe, D.L. Costill., *Disproportionate loss of thin filaments in human soleus muscle after 17-day bed rest.* Muscle & Nerve, 1998. 21: p. 1280-1289.

17. Riley, D.A., J.L.W. Bain, J.L. Thompson, R.H. Fitts, J.J. Widrick, S.W. Trappe, T.A. Trappe, and D.L. Costill., *Decreased thin filament density and length in human atrophic soleus muscle fibers after spaceflight.* J. Appl. Physiol., 2000. 88: p. 567-572.

18. Riley, D.A., S. Ellis, G.R. Slocum, T. Satyanaranyana, J.L.W. Bain, and F.R. Sedlak., *Morphological and biochemical changes in soleus and extensor digitorum longus muscles of rats orbited in spacelab 3.* The Physiologist., 1985. 28(6): p. S207-S208.

19. Riley, D.A., S. Ellis, G.R. Slocum, T. Satyanaryana, J.L.W. Bain, and F.R. Sedlak., *Hypogravity-induced atrophy of rat soleus and extensor digitorum longus muscles.* Muscle & Nerve, 1987. 10: p. 560-568.

20. Riley, D.A., G.R. Slocum, J.L.W. Bain, F.R. Sedlak, T.E. Sowa, and J.W. Mellender, *Rat hindlimb unloading: soleus histochemistry, ultrastructure, and electromyography.* J. Appl. Physiol., 1990. 69(1): p. 58-66.

21. Riley, D.A., E.I. Ilyina-Kakueva, S. Ellis, J.L.W. Bain, G.R. Slocum, and F.R. Sedlak, *Skeletal muscle fiber, nerve and blood vessel breakdown in space-flown rats.* FASEB J., 1990. 4: p. 84-91.

22. Riley, D.A., E. Ellis, C.S. Giometti, J.F.Y. Hoh, E.I. Ilyina-Kakueva, V. Oganov, G.R. Slocum, J.L.W. Bain, and F.R. Sedlak, *Muscle sarcomere lesion and thrombosis after spaceflight and suspension unloading.* J. Appl. Physiol., 1992. 73: p. S33-S43.

23. Thompson, J.L., E.M. Balog, R.H. Fitts, and D.A. Riley., *Five myofbrillar lesion types in eccentrically challenged, unloaded rat adductor longus muscle -- a test model.* Anat. Rec., 1999. 254: p. 39-52.

24. Vijayan, K., J.L. Thompson, and D.A. Riley, *Sarcomere lesion damage occurs mainly in slow fibers of reloaded rat adductor longus muscles.* J. Appl. Physiol., 1998. 85(3): p. 1017-1023.

25. Kasper, C.E., *Sarcolemmal disruption in reloaded atrophic skeletal muscle.* J. Appl. Physiol., 1995. 79(2): p. 607-614.

26. Vijayan, K., J.L. Thompson, K.M. Norenberg, R.H. Fitts, and D.A. Riley., *Fiber-type susceptibility to eccentric contraction-induced damage of hindlimb-unloaded rat AL muscles.* J. Appl. Physiol., 2001. 90: p. 770-776.

27. Kirby, C.R., M.J. Ryan, and F.W. Booth, *Eccentric exercise training as a countermeasure to non-weight -bearing soleus muscle atrophy.* J. Appl. Physiol., 1992. 73(5): p. 1894-1899.

28. Krippendorf, B.B., and D.A. Riley, *Distinguishing unloading- versus reloading-induced changes in rat soleus muscle.* Muscle & Nerve, 1993. 16: p. 99-108.

29. Krippendorf, B.B., and D.A. Riley, *Temporal changes in sarcomere lesions of rat adductor longus muscles during hindlimb reloading.* Anat. Rec., 1994. 238: p. 304-310.

30. Prisby, R.D., R.A. Reyes, J.M. Eason and A.G. Nelson. *Criteria for assessing skeletal muscle damage from electron micrographs*. in *American College of Sports Medicine*. 2001. Baltimore, MD.

31. Prisby, R.D., and A.G. Nelson, *Glucose-6-phosphate dehydrogenase activity is increased after 7-d of hindlimb suspension unloading and 16 h of reloading.* Med. Sci. Sports Exerc., 2002. 34(5): p. S22.

32. Anzil, A.P., G. Sancesario, R. Massa, and G. Bernard, *Myofibrillar disruption in the rabbit soleus muscle after one-week hindlimb suspension.* Muscle and Nerve, 1991. 14: p. 358-369.

33. Sancesario, G., R. Massa, A.P. Anzil, and G. Bernardi., *Active muscle length reduction progressively damages soleus in hindlimb-suspended rabbits.* Muscle and Nerve, 1992. 15: p. 1002-1015.

34. Fitts, R.H., D.A. Riley, and J.J. Widrick., *Invited review: microgravity and skeletal muscle.* J. Appl. Physiol., 2000. 89: p. 823-839.

35. Riley, D.A., J.L Thompson, B.B. Krippendorf, and G.R. Slocum, *Review of spaceflight and hindlimb suspension unloading induced sarcomere damage and repair.* Basic Appl. Myology, 1995. 5: p. 139-145.

36. Warren, G.L., D.A. Hayes, D.A. Lowe, J.H. Williams, and R.B. Armstrong, *Eccentric contraction-induced injury in normal and hindlimb-suspended mouse soleus and EDL muscles.* J. Appl. Physiol., 1994. 77: p. 1421-1430.

37. Armstrong, R.B., R.W. Ogilvie, and J.A. Schwane, *Eccentric exercise-induced injury to rat skeletal muscle.* J. Appl. Physiol., 1983. 54: p. 80-93.

38. Lieber, R.L.a.J.F., *Morphologic and mechancial basis of delayed-onset muscle soreness.* J. Am. Acad. Orthop. Surg., 2002. 10: p. 67-73.

39. MacIntyre, D.L., W.D. Reid, and D.C. McKenzie., *Delayed muscle soreness: the inflammatory response to muscle injury and its clinical implications.* Sports Med., 1995. 20(1): p. 24-40.

40. Komulainen, J., J. Kytola, and V. Vihko., *Running-induced muscle injury and myocellular enzyme release in rats.* J. Appl. Physiol., 1994. 77(5): p. 2299-2304.

41. Fridén, J., *Muscle soreness after exercise: implications of morphological changes.* Int. J. Sports med., 1984. 5: p. 57-66.

42. Ebbeling, C.B., and P. M. Clarkson, *Exercise-induced muscle damage and adaptation.* Sports Med., 1989. 7: p. 207-234.

43. Fridén, J., M. Sjöström, and B. Ekblom, *A morphological study of delayed muscle soreness.* Experientia, 1980. 37: p. 506-507.

44. Fridén, J., M. Sjostrom, and B. Ekblom., *Myofibrillar damage following intense eccentric exercise in man.* Int. J. Sports Med., 1983. 4: p. 170-176.

45. Ilyina-Kakueva, E.I., V.V. Portugalov, and N.P. Kirvenkova, *Space flight effects on the skeletal muscle of rats.* Aviat. Space Environ. Med., 1976. 47: p. 700, 703.

46. Fridén, J., R.L. Lieber., *Segmental muscle fiber lesions after repetitive eccentric contractions.* Cell Tissue Res., 1998. 293: p. 165-171.

47. Ogilvie, R.W., R.B. Armstrong, K.E. Baird, and C.L. Bottoms., *Lesions in the rat soleus muscle following eccentrically biased exercise.* Am. J. Anat., 1988. 182: p. 335-346.

48. D'Amelio, F., and N.G. Daunton., *Effects of spaceflight in the adductor longus muscle of rats flown in the soviet biosatellite cosmos 2044. a study employing neural cell adhesion molecule (n-cam) immunocytocheistory and convential morphological techniques (light and electron microscopy).* J. Neuropath. Exp. Neurol., 1992. 51(4): p. 415-431.

49. Fridén, J., U. Kjorell, and L.-E. Thornell., *Delayed muscle soreness and cytoskeletal alterations: an immunocytological study in man.* Int. J. Sports Med., 1984. 5: p. 15-18.

50. Clarkson, P.M., and I. Tremblay, *Exercise-induced muscle damage, repair and adaptation in humans.* J. Appl. Physiol., 1988. 65(1): p. 1-6.

51. Schwane, J.A.a.R.B.A., *Effects of training on skeletal muscle injury from downhill running in rats.* J. Appl. Physiol., 1983. 55(3): p. 969-975.

52. Nosaka, K., and P.M. Clarkson., *Muscle damage following repeated bouts of high force eccentric exercise.* Med. Sci. Sports Exerc., 1995. 27(9): p. 1263-1269.

53. Golden, C.L., and G.A. Dudley, *Strength after bout of eccentric or concentric actions.* Med. Sci. Sports Exerc., 1992. 24(8): p. 926-933.

54. Ebbeling, C.B.a.P.M.C., *Muscle adaptation prior to recovery following eccentric exercise.* Eur. J. Appl. Physiol., 1990. 60: p. 26-31.

55. Newham, D.J., D.A. Jones, and P.M. Clarkson, *Repeated high-force eccentric exercise: effects on muscle pain and damage.* J. Appl. Physiol., 1987. 63(4): p. 1381-1386.

56. Fridén, J., *Changes in human skeletal muscle induced by long-term eccentric exercise.* Cell Tissue Res., 1984. 236: p. 365-372.

57. Warren, G.L., D.A. Lowe, D.A. Hayes, C.J. Karwoski, B.M. Prior and R.B. Armstrong, *Excitation failure in eccentric contraction-induced injury of mouse soleus muscle.* J. Physiol., 1993. 468: p. 487-499.

58. Balnave, C.D., and D.G. Allen, *Intracellular calcium and force in single muscle fibers following repeated contractions with stretch.* J. Physiol. (Lond), 1995. 488(1): p. 25-36.

59. Pizza, F.X., B.J. Davis, and S.D. Hendrickson., *Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression.* J. Appl. Physiol., 1996. 80(1): p. 47-55.

60. Lieber, R.L., C.B. Johansson, H.L. Vahlsing, A.R. Hargens, and E.R. Feringa, *Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. contractile properties.* Exp. Neurol., 1986. 91: p. 423-434.

61. Lieber, R.L., J.O. Friden, A.R. Hargens, and E.R. Feringa., *Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. II. Morphometric properties.* Exp. Neurol., 1986. 91: p. 435-448.

62. Morey, E.R., *Spaceflight and bone turnover: correlation with a new rat model of weightlessness.* Bioscience, 1979. 29: p. 168-172.

63. Lieber, R.L., *Skeletal Muscle Structure and Function: Implications for Rehabilitation and Sports Medicine*, ed. J.P. Butler. 1992, Baltimore: Williams & Wilkens. 303.

64. Tischler, M.E., E.J. Henriksen, S.R. Jaspers, S. Jacob and C. Kirby, *Changes in muscles accompanying non-weight-bearing and weightlessness*, in *Adances in Myochemistry*, G. Benzi, Editor. 1989, John Libbey Eurotext Ltd. p. 325-338.

65. Jaspers, S.R., and M.E. Tischler, *Atrophy and growth failure of rat hindlimb muscles in tail-cast suspension.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 1984. 57(5): p. 1472-1479.

66. Williams, P.E., and G. Goldspink., *Connective tissue changes in immobilized muscle.* J. Anat. (London), 1984. 138: p. 343-350.

67. Wilkerson, M.K., J. Muller-Delp, P.N. Colleran, and M.D. Delp., *Effects of hindlimb unloading on rat cerebral, splenic, and mesenteric resistance artery morphology.* J. Appl. Physiol., 1999. 87(6): p. 2115-2121.

68. Park, E., and E. Schultz., *A simple hindlimb suspension apparatus.* Aviat. Space Environ. Med., 1993. 64: p. 401-404.

69. Morey-Holton, E.R., and R.K. Globus, *Hindlimb unloading of growing rats: a model of predicting skeletal changes during space flight.* Bone, 1998. 22(5): p. 83S-88S.

70. Herbert, M.E., R.R. Roy, and V.R. Edgerton., *Influence of one-week hindlimb suspension and intermittent high-load exercise on rat muscle.* Exp. Neur., 1988. 102: p. 190-198.

71. Armstrong, R.B., G.L. Warren, and J.A. Warren., *Mechanisms of exercise-induced muscle fibre injury.* Sports Med., 1991. 12(3): p. 184-207.

72. Clarkson, P.M., W.C. Byrnes, E. Gillison, and E. Harper, *Adaptation to exercise-induced muscle damage.* Clin. Sci., 1987. 73: p. 383-386.

73. Tullson, P., and R.B. Armstrong, *Muscle hexose monophosphate shunt activity following exercise.* Experentia, 1981. 37: p. 1311-1312.

74. Hauschka, E.O., R.R. Roy, and V.R. Edgerton., *Periodic weight support effects on rat soleus fibers after hindlimb suspension.* J. Appl. Physiol., 1988. 65(3): p. 1231-1237.

75. Graham, S.C., R.R. Roy, S.P. West, D. Thomason and K.M. Baldwin, *Exercise effects on the size of metabolic properties of*

soleus fibers in hindlimb-suspended rats. Aviat. Space Environ. Med., 1989. 60: p. 226-34.

76. Michel, R.N., and P.F. Gardiner., *To what extent is hindlimb suspension a model of disuse?* Muscle & Nerve., 1990. 13: p. 646-653.

77. Oganov, V.S., L.M. Murashoko, O.E. Kabitskaya, T. Szilanyi and M. Rapcsak, *Physiological characteristics of rat skeletal muscles after the flight on board "Cosmos-2044: biosatellite.* The Physiologist, 1991. 34: p. S174-S176.

78. Stevens, L., and Y. Mounier, *Functional properties of soleus and edl muscles after weightlessness (Cosmos 2044).* The Physiologist, 1991. 34: p. S172-S173.

79. Jiang, B., Y. Ohira, R.R. Roy, Q. Nguyen, E.I. Ilyina-Kakueva, V. Oganov and V.R. Edgerton, *Adaptation of fibers in fast-twitch muscles in rats to spaceflight and hindlimb suspension.* J. Appl. Physiol., 1992. 73(2): p. 58S-65S.

80. Musacchia, X.J., J.M. Steffen, R.D. Fell, M.J. Dombrowski, V.W. Oganov & E.I. Ilyina-kakueva, *Skeletal muscle atrophy in response to 14 days of weightlessness: vastus medialis.* J. Appl. Physiol., 1992. 73(2): p. 44S-50S.

81. Pottle, D., and L.E. Gosselin, *Impact of mechanical load on functional recovery after muscle reloading.* Med. Sci. Sports Exerc., 2000. 32(12): p. 2012-2017.

82. Gazenko, O.G., Ye.A. Ilyin, Y.A. Savina, L.V. Serova, A.S. Kaplanskiy, V.S. Oganov, I.A. Popova, K.V. Smirnov, I.V. Konstantinova., *Experiments with rats flown aboard Cosmos-1667 biosatellite (main objectives, conditions and results).* Kosmicheskaya Biologiya i Aviakosmicheskaya Meditsina, 1987. 21(4): p. 8-16.

83. Gazenko, O.G., A.I. Grigoriev, and I.B. Kozlovskaya, *Mechanisms of acute and chronic effects of microgravity.* The Physiologist, 1987. 30(1): p. S1-S5.

84. Edgerton, V.R., M.-Y. Zhou, Y. Ohira, H. Klitgaard, B. Jiang, G. Bell, B. Harris, B. Saltin, P.D. Gollnick, R.R. Roy, M.K. Day, and M. Greenisen, *Human fiber size and enzymatic properties after 5 and 11 days of spaceflight.* J. Appl. Physiol., 1995. 78: p. 1733-1739.

85. Riley, D.A., *Is skeletal muscle ready for longterm spaceflight and return to gravity?* Adv. Space Biol. Med., 1999. 7: p. 31-48.

86. Russell, B., D.J. Dix, D.L. Haller, and J. Jacobs-El., *Repair of injured skeletal muscle: a molecular approach.* Med. Sci. Sports Exerc., 1992. 24(2): p. 189-196.

87. Gibala, M.J., J. D. MacDougall, M.A. Tarnopolsky, W.T. Stauber, and A. Elorriaga., *Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise.* J. Appl. Physiol., 1995. 78(2): p. 702-708.

88. MacPherson, P.C.D., M.A. Schork, and J.A. Faulkner., *Contraction-induced injury to single fiber segments from fast and slow muscles of rats by single stretches.* Am. J. Physiol., 1996. 271(Cell Physiol. 40): p. C1438-C1446.

89. McHugh, D., D. Connolly, R. Eston, and G. Glein., *Exercise-induced muscle damage and potential mechanisms for the repeated bout effect.* Sports Med., 1999. 27: p. 157-70.

90. Powers, S.K., and E.T. Howley, *Exercise Physiology: Theory and Application to Fitness and Performance*. 4th ed. 2001, Boston: McGraw-Hill. 540.

91. Mair, J., M. Mayr., E. Muller, A. Koller, C. Haid, E. Artner-Dworzak, C. Calzolari, C. Larve, and B. Puschendorf., *Rapid adaptation to eccentric exercise-induced muscle damage.* Int. J. Sports Med., 1994. 16(6): p. 352-356.

92. Armstrong, R., *Mechanisms of exercise-induced delayed onset muscular soreness: a brief review.* Med Sci. Sports Exerc., 1984. 16: p. 529-538.

93. Stauber, W.T., *Eccentric action of muscles: physiology, injury and adaptation.* Exerc. Sports Sci. Rev., 1989. 17: p. 157-185.

94. Fridén, J., and R.L. Lieber., *Structural and mechanical basis of exercise-induced muscle injury.* Med. Sci. Sports Exerc., 1992. 24(5): p. 521-530.

95. Koh, T.J., and S.V. Brooks., *Lengthening contractions are not required to induce protection from contraction-induced muscle injury.*

Am. J. Physiol. Regulatory Integrative Comp. Physiol., 2001. 281: p. R155-R161.

96. Morgan, D.L., *New insight into the behavior of muscle during active lengthening.* Biophys. J., 1990. 57: p. 209-221.

97. Baldwin, K.M., V.J. Caiozzo, F. Haddad, M.J. Baker, and R.E. Herrick., *The effects of space flight on the contractile apparatus of antigravity muscles: implications for aging and deconditioning.* J. Gravitational Physiol., 1994. 1(1): p. 8-11.

98. Baranski, S., W. Baranska, M. Marciniak, and E.I. Ilyina-Kakueva, *Ultrasonic ("ultrastructural") investigations of the soleus muscle after space flight on the Biosputnik 936. Aviat Space Environ Med*, 1979. 50: p. 930-934.

99. Bodine-Fowler, S.C., R.R. Roy, W. Rudolph, N. Haque, I.B. Kozlovskaya, and V.R. Edgerton., *Spaceflight and growth effects on muscle fibers in the rhesus monkey.* J. Appl. Physiol., 1992. 73(2): p. 82S-89S.

100. Day, M.K., D.L. Allen, L. Mohajerani, M.C. Greenisen, R.R. Roy, and V.R. Edgerton., *Adaptations of human skeletal muscle fibers to spaceflight.* J. Gravitat. Physiol., 1995. 2(1): p. P47-P50.

101. Desplanches, D., M.H. Mayet, E.I. Ilyina-Kakueva, B. Sempore, R. Flandrois, *Skeletal muscle adaptation in rats flown on cosmos 1667.* J. Appl. Physiol., 1990. 68(1): p. 48-52.

102. Grindeland, R.E., R.W. Ballard, J.P.Connolly, and M.F. Vasques, *Cosmos 2044 mission.* J. Appl. Physiol., 1992. 73(Suppl.): p. 1S-3S.

103. Ohira, Y., B. Jiang, R.R. Roy, V Ogranov, E. Ilyina-Kakueva, J.F. Marini, and V.R. Edgerton., *Rat soleus muscle fiber responses to 14 days of spaceflight and hindlimb suspension.* J. Appl. Physiol., 1992. 73(2): p. 51S-57S.

104. Midrio, M., D. Danieli Betto, R. Betto, D. Noventa, and F. Antico, *Cordotomy-denervation interactions on contractile and myofibrillar properties of fast and slow muscles in the rat.* Exp. Neurol., 1988. 100: p. 216-236.

105. Dudley, G.A., M.J. Castro, S. Rogers, and D.G. Apple, Jr., *A simple means of increasing muscle size after spinal cord injury: a pilot study.* Eur. J. Appl. Physiol., 1999. 80: p. 394-396.

106. Finol, H.J., D.M. Lewis, and R. Owens, *The effects of denervation on contractile properties of rat skeletal muscle.* J. Physiol., 1981. 319: p. 81-92.

107. Haida, N., W.M. Fowler, Jr., R.T. Abresch, D.B. Larson, R.B. Sharman, R.G. Taylor, and R.K. Entrikin., *Effect of hind-limb suspension on young and adult skeletal muscle I: normal mice.* Exp. Neur., 1989. 103: p. 68-76.

108. Hauschka, E.O., R.R. Roy and V.R. Edgerton., *Size and metabolic properties of single muscle fibers in rat soleus after hindlimb suspension.* J. Appl. Physiol., 1987. 62: p. 1231-1237.

109. Steffen, J.M., R.D. Fell, T.E. Geoghegan, L.C. Ringel, and X.J. Musacchia, *Age effects on rat hindlimb muscle atrophy during suspension unloading.* J. Appl. Physiol., 1990. 68(3): p. 927-931.

110. Tischler, M.E., E.J. Henirksen, K.A. Munoz, C.S. Stump, C.R. Woodman, and C.R. Kirby, *Spaceflight on STS-48 and earth-based unweighting produce similar effects on skeletal muscle of young rats.* J. Appl. Physiol., 1993. 74: p. 2161-2165.

111. Hornberger, T.A., R.B. Hunter, S.C. Kandarian, and K.A. Esser., *Regulation of translation factors during hindlimb unloading and denervation of skeletal muscle in rats.* Am. J. Physiol. Cell Physiol., 2001. 281: p. C179-C187.

112. Edgerton, V.R., and R.R. Roy, *Neuromuscular adaptation to actual and simulated weightlessness.*, in *Advances in Space Biology and Medicine*, S.L. Bonting, Editor. 1994, JAI Press: Greenwich, Conn. p. 33-67.

113. Fitts, R.H., J.J. Widrick, S.T. Knuth, C.A. Blaser, M. Karhanek, S.W. Trappe, T.A. Trappe and D.L. Costill, *Force-velocity and force-power properties of human muscle fiber after spaceflight.* Med. Sci. Sports Exerc., 1998. 29: p. S190-S192.

114. Riley, D.A., *Review of primary spaceflight-induced and secondary reloading-induced changes in slow antigravity muscles of rats.* Adv. Space Res., 1998. 21(8/9): p. 1073-1075.

115. Stevens, L., F. Picquet, M-P Catinot, and Y. Mounier., *Differential adaptation to weightlessness of functional and structural characteristics of rat hindlimb muscles.* J. Gravitational Physiol., 1996. 3(2): p. 54-57.

116. Stein, T.P., and T. Gaprindashvili, *Spaceflight and protein metabolism with special reference to humans.* Am. J. Clin. Nutr., 1994. 60: p. 806S-819S.

117. Esser, K.A., and T.P. White., *Mechanical loads affects growth and maturation of skeletal muscle grafts.* J. Appl. Physiol., 1995. 78: p. 30-37.

118. Fridén, J., and R.L. Lieber, *Muscle damage induced by cyclic eccentric contraction: biochemical and structural studies*, in *Muscle Damage*, S. Salmons, Editor. 1997, Oxford University Press: Oxford. p. 41-63.

119. Dop Bär, P.R., J.C. Reijneveld, J.H.J. Wokke, S.C.J.M. Jacobs, and A.L. Bootsma, *Muscle damage induced by exercise: nature, prevention and repair*, in *Muscle Damage*, S. Salmons, Editor. 1997, Oxford University Press: Oxford. p. 1-27.

120. Stauber, W.T., V.K. Fritz, T.E. Burkovskaya, and E.I. Ilyina-Kakueva., *Effect of injury on mast cells of rat gastrocnemius muscle with respect to gravitational exposure.* Exp. Mol. Pathol., 1993. 59: p. 87-94.

121. Lieber, R.L., M.C. Schmitz, D.K. Mishra, and J. Friden., *Contractile and cellular remodeling in rabbit skeletal muscle after cyclic eccentric contractions.* J. Appl. Physiol., 1994. 77(4): p. 1926-1934.

122. Beaconsfield, P., *Local metabolic response to physio-pathological demands: the pentose phosphate pathway.* Experientia, 1963. 8: p. 437-438.

123. Rifenderick, D.H., C.L. Koski, and S.R. Max, *Metabolic studies of skeletal muscle regeneration.* Exp. Neur., 1974. 45: p. 527-540.

124. Stipanuk, M.H., *Biochemical and physiological aspects of human nutrition*. 2000, Philadelphia: W.B. Saunders Company. 1007.

125. Jennings, R.T., and J.P. Bagian, *Musculoskeletal injury review in the U.S. space program.* Aviat. Space Environ. Med., 1996. 67: p. 762-6.

126. Greenleaf, J.E., R. Bulbulian, E.M. Bernauer, W.L. Haskell, and T. Moore., *Exercise-training protocols for astronauts in microgravity.* J. Appl. Physiol., 1989. 67(6): p. 2191-2204.

127. Klein, K.E., H.M. Wegmann, and P. Kuklinski., *Athletic endurance training-advantage for space flight?: the significance of physical fitness for selection and training of Spacelab crews.* Aviat. Space Environ. Med., 1977. 48: p. 215-222.

128. Convertino, V.A., *Exercise and adaptation to microgravity environments.*, in *Handbook of Physiology:Section 4: Environmental Physiology*, M.J. Fregly, and C.M. Blatteis, Editor. 1996, Oxford University Press: New York. p. 815-843.

129. Stegemann, J., A. Busert, and D. Brock, *Influence of fitness on the blood pressure control system in man.* Aerospace Med., 1974. 45: p. 45-48.

130. Mangseth, G.R., and E.M. Bernauer, *Cardiovascular response to tilt in endurance trained subjects exhibiting syncope reactions.* Med. Sci. Sports Exerc., 1980. 12: p. 140.

131. Goldwater, D.J., M. DeLada, A. Polese, L. Keil, and J.A. Luetscher, *Effect of athletic conditioning on orthostatic tolerance after prolonged bedrest.* Circulation., 1980. 62III: p. 287.

132. Raven, P.B., D. Rohm-Young, and C.G. Blomqvist, *Physical fitness and cardiovascular response to lower body negative pressure.* J. Appl. Physiol., 1984. 56: p. 138-144.

133. Tipton, C.M., R.D. Matthes, and T.G. Bedford, *Influence of training on the blood pressure changes during lower body negative pressure in rats.* Med. Sci. Sports Exerc., 1982. 14: p. 81-90.

134. Saltin, B., G. Blomqvist, J.H. Mitchell, R.L. Johnson, K. Wildenthal, and C.B. Chapman., *Response to exercise after bed rest and after training.* Circulation, 1968. 38(Suppl. 7): p. 1-78.

135. Buckley, J.C., L.D. Lane Jr., B.D. Levine, D.E. Watenpaugh, S.J. Wright, W.E. Moore, F.A. Gaffney, and C.G. Blomqvist., *Orthostatic intolerance after spaceflight.* J. Appl. Physiol., 1996. 81: p. 7-18.

136. Tipton, C., *Considerations for exercise prescription in future space flights.* Med. Sci. Sports Exerc., 1983. 15(5): p. 441-444.

137. Fitts, R.H., D.R. Riley, and J.J. Widrick., *Functional and structural adaptations of skeletal muscle to microgravity.* J. Exp. Biol., 2001. 204: p. 3201-3208.

138. Hoppeler, H., B. Kayser, S.L. Lindstedt, C. Boesch, M. Kushmerick, A. Draeger, and F. Booth., *Recommendations for muscle research in space.* Int. J. Sports Med., 1997. 18(Suppl. 4): p. S280-S282.

139. Tesch, P.A., and H.E. Berg, *Effects of spaceflight on muscle.* J. Gravitational Physiol., 1998. 5(1): p. P19-P23.

140. Globus, R.K., D.D. Bikle, B. Halloran, and E.R. Morey-Holton., *The temporal response of bone to unloading.* Endocrinology, 1986. 118: p. 733-742.

141. Musacchia, X.J., J.M. Steffen, R.D. Fell, and M.J. Dombrowski., *Skeletal muscle response to spaceflight, whole body suspension, and recovery in rats.* J. Appl. Physiol., 1990. 69(6): p. 2248-2253.

142. McCall, G.E., W.C. Brynes, A.L. Dickinson, and S.J. Fleck, *Sample size required for the accurate determination of fiber area and capillarity of human skeletal muscle.* J. Appl. Physiol., 1998. 23(6): p. 594-599.

143. Franks, B.D., and S.W. Hucks, *Why does everyone use the .05 significance level?* Res. Quart. Ex. Sport, 1986. 56: p. 245-249.

144. Wronski, T.J., and E.R. Morey-Holton, *Skeletal response to simulated weightlessness: a comparison of suspension techniques.* Aviat. Space Environ. Med., 1987. 58(63-68).

145. Portugalov, V.V., E.A. Savina, A.S. Kaplansky, V.I. Yakovleva, G.I. Plakhuta-Plakutina, A.S. Pankova, P.I. Katunya, M.G. Subich, and S.A. Buvalio., *Effect of spaceflight factors on the mammal: experimental morphological study.* Aviat. Space Environ. Med., 1976. 47: p. 813-816.

146. Mason, J.W., *Corticosteriod response to chair restraint in the monkey.* Am. J. Physiol., 1972. 222: p. 1291-1294.

147. Ingalls, C.P., J.C. Wenke, and R.B. Armstrong., *Time course changes in [Ca^{2+}]i, force protein content in hindlimb-suspended mouse soleus muscles.* Aviat. Space Environ. Med., 2001. 72(471-476).

148. Armstrong, J.W., K. Nelson, S.S. Simske, M.W. Luttges, J.J. Iandolo, and S.K. Chapes., *Skeletal unloading causes organ-specific changes in immune cell response.* J. Appl. Physiol., 1993. 75: p. 2734-2739.

149. Tabary, J.C., C. Tabary, C. Tardieu, G. Tardieu, and G. Goldspink, *Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster cast.* J. Physiol. (Lond)., 1972. 224: p. 231-244.

150. Evan, W.J., and J.G. Cannon., *The metabolic effects of exercise-induced muscle damage.* Exerc. Sport Sci. Rev., 1991. 19: p. 99-125.

151. Smith, L.L., *Acute inflammation: the underlying mechanism in delayed onset muscle soreness?* Med. Sci. Sports Exerc., 1991. 20: p. 345-353.

152. Widrick, J.J., S.T. Knuth, K.M Norenberg, J.G. Romatowski, J.L.W. Bain, D.A. Riley, M. Karhanek, S.W. Trappe, T.A. Trappe, D.L. Costill, and R.H. Fitts, *Effects of a 17-day spaceflight on contractile properties of human soleus muscle fibres.* J. Appl. Physiol., 1999. 516: p. 915-930.

153. Bigard, Z.X., D. Merino, F. Lienhard, and B. Serrurier., *Muscle damage induced by running training during recovery from hindlimb suspension: the effect of dantrolene sodium.* Eur. J. Appl. Physiol., 1997. 76: p. 421-427.

154. Bigard, A.X., D. Merino, F. Lienhard, and B. Serrurier., *Quantitative assessment of degenerative changes in soleus muscle after hindlimb suspension and recovery.* Eur. J. Appl. Physiol., 1997. 75: p. 380-387.

155. McArdle, A., and M.J. Jackson., *Intracellular mechanisms involved in skeletal muscle damage.*, in *Muscle Damage*, S. Salmons, Editor. 1997, Oxford University Press: Oxford. p. 90-106.

156. Benoit, P.W., and W.D. Belt., *Destruction and regeneration of skeletal muscle after treatment with a local anesthetic, bupivacaine (Marcaine).* J. Anat., 1970. 107: p. 547-556.

157. Wagner, K.R., F.C. Kaufman, and S.R. Max, *The pentose phosphate pathway in regenerating skeletal muscle.* Biochem. J., 1978. 170: p. 17-22.

158. Thomason, D.B., and F.W. Booth., *Protein metabolism and β– myosin heavy-chain mRNA in unweighted soleus muscle.* Am. J. Physiol. Regulatory Integrative Comp. Physiol., 1989. 257: p. R300-R305.

159. Newham, D.J., G. McPhail, K.R. Mills and R.H.T. Edwards., *Ultrastructural changes after concentric and eccentric contractions of human muscle.* J. Neurol. Sci., 1983. 61: p. 109-122.

APPENDIX A. BODY WEIGHT DATA

Table A.1. Initial body weights.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	210	1	HSU	2	196
3	ExHSU	1	188	4	HSU	2	180
7	ExHSU	1	145	6	HSU	2	155
10	ExHSU	1	142	13	HSU	2	166
22	ExHSU	1	206	23	HSU	2	198
28	ExHSU	1	217	30	HSU	2	212
33	ExHSU	1	213	31	HSU	2	208
34	ExHSU	1	190	32	HSU	2	201
41	ExHSU	1	222	36	HSU	2	209
45	ExHSU	1	205	38	HSU	2	210
46	ExHSU	1	200	43	HSU	2	203
49	ExHSU	1	224	47	HSU	2	220
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	142	5	C	4	170
12	Ex	3	198	9	C	4	156
14	Ex	3	168	15	C	4	188
17	Ex	3	148	16	C	4	159
20	Ex	3	214	18	C	4	138
24	Ex	3	189	19	C	4	132
25	Ex	3	223	21	C	4	213
29	Ex	3	213	26	C	4	233
37	Ex	3	210	27	C	4	218
42	Ex	3	224	35	C	4	225
44	Ex	3	209	39	C	4	234
48	Ex	3	224	40	C	4	224

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.

Table A.2. Pre-Suspension body weights.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	241	1	HSU	2	241
3	ExHSU	1	233	4	HSU	2	219
7	ExHSU	1	177	6	HSU	2	194
10	ExHSU	1	194	13	HSU	2	209
22	ExHSU	1	215	23	HSU	2	212
28	ExHSU	1	217	30	HSU	2	224
33	ExHSU	1	220	31	HSU	2	220
34	ExHSU	1	204	32	HSU	2	217
41	ExHSU	1	224	36	HSU	2	215
45	ExHSU	1	207	38	HSU	2	214
46	ExHSU	1	202	43	HSU	2	208
49	ExHSU	1	225	47	HSU	2	223
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	185	5	C	4	221
12	Ex	3	245	9	C	4	200
14	Ex	3	229	15	C	4	230
17	Ex	3	198	16	C	4	199
20	Ex	3	227	18	C	4	190
24	Ex	3	205	19	C	4	187
25	Ex	3	226	21	C	4	224
29	Ex	3	225	26	C	4	231
37	Ex	3	210	27	C	4	232
42	Ex	3	230	35	C	4	228
44	Ex	3	211	39	C	4	234
48	Ex	3	229	40	C	4	222

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.

Table A.3. Body Weight across time for the ExHSU group.

T	Rat #											
	2	3	7	10	22	28	33	34	41	45	46	49
1	210	203	151	156	206	221	219	210	219	203	196	222
2	209	204	155	164	211	220	219	200	218	206	196	215
3	217	216	163	173	210	222	216	200	220	207	197	220
4	222	216	163	178	213	223	223	204	224	206	202	218
5	226	225	163	181	211	221	222	204	224	204	202	222
6	241	233	177	194	215	217	220	204	224	207	202	225
7	235	236	170	189	198	220	243	201	230	209	208	230
8	242	218	169	192	222	217	223	214	229	209	208	213
9	230	229	174	187	212	196	216	200	219	203	198	214
10	230	236	178	185	214	215	216	200	224	207	194	219
11	213	222	170	187	214	202	203	200	220	200	198	222

T = Time points.

Table A.4. Body weight across time for the HSU group.

T	Rat #											
	1	4	6	13	23	30	31	32	36	38	43	47
1	203	186	161	200	206	221	220	208	203	212	207	219
2	207	193	169	201	208	218	219	212	205	211	203	213
3	214	203	179	197	210	208	210	198	211	215	210	223
4	217	207	179	195	211	225	223	215	210	217	213	217
5	225	212	186	204	213	227	223	216	210	216	209	216
6	241	219	194	209	212	224	220	217	215	214	208	223
7	231	214	199	191	209	239	226	231	216	222	216	227
8	235	214	210	201	213	235	232	219	222	224	218	217
9	200	211	209	193	195	225	227	200	198	220	206	206
10	216	202	214	201	200	217	227	211	204	218	205	216
11	215	202	215	200	200	196	206	198	204	212	210	217

T = Time points.

Table A.5. Body weight across time for the Ex group.

T	Rat #											
	11	12	14	17	20	24	25	29	37	42	44	48
1	153	204	182	154	226	195	228	225	204	227	213	228
2	162	210	180	168	226	201	230	229	200	220	204	223
3	171	225	220	175	228	200	228	228	206	230	213	227
4	170	231	216	182	230	204	232	228	205	228	211	227
5	177	238	226	185	228	201	229	224	206	229	207	226
6	185	245	229	198	227	205	226	225	210	230	211	229
7	185	239	222	192	237	208	235	233	214	235	216	231
8	187	250	230	191	239	213	235	234	213	235	217	235
9	192	257	226	200	239	210	238	238	209	237	219	236
10	195	262	232	205	243	215	242	239	214	241	223	242
11	198	263	235	203	243	215	242	239	222	244	225	242

T = Time points.

Table A.6. Body weight across time for the control group.

T	Rat #											
	5	9	15	16	18	19	21	26	27	35	39	40
1	180	171	185	175	154	149	220	232	228	223	233	227
2	189	175	196	174	162	157	223	234	227	222	229	224
3	197	188	210	187	174	169	221	231	231	227	236	227
4	204	188	212	190	179	173	224	235	231	229	241	228
5	209	189	220	194	184	178	222	234	230	228	235	225
6	221	200	230	199	190	187	224	231	232	228	234	222
7	214	199	226	198	186	184	229	241	233	236	238	238
8	213	203	238	209	195	189	237	243	243	235	233	231
9	224	210	239	209	197	194	233	245	241	235	236	237
10	226	212	246	214	204	198	238	248	242	238	234	236
11	226	198	251	215	206	199	238	248	242	236	238	235

T = Time points.

Table A.7. Body weights at Sacrifice.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	213	1	HSU	2	215
3	ExHSU	1	222	4	HSU	2	[redacted]
7	ExHSU	1	178	6	HSU	2	215
10	ExHSU	1	187	13	HSU	2	200
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	202	30	HSU	2	196
33	ExHSU	1	203	31	HSU	2	206
34	ExHSU	1	[redacted]	32	HSU	2	198
41	ExHSU	1	220	36	HSU	2	204
45	ExHSU	1	200	38	HSU	2	212
46	ExHSU	1	198	43	HSU	2	210
49	ExHSU	1	[redacted]	47	HSU	2	217
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	198	5	C	4	226
12	Ex	3	263	9	C	4	178
14	Ex	3	235	15	C	4	251
17	Ex	3	203	16	C	4	215
20	Ex	3	243	18	C	4	206
24	Ex	3	215	19	C	4	199
25	Ex	3	242	21	C	4	238
29	Ex	3	239	26	C	4	248
37	Ex	3	222	27	C	4	242
42	Ex	3	244	35	C	4	236
44	Ex	3	225	39	C	4	238
48	Ex	3	242	40	C	4	235

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise; C, control.
Note: Blackened numbers represent data excluded from analysis.

APPENDIX B. MUSCLE WEIGHT DATA

Table B.1. Soleus wet weights.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	0.0896	1	HSU	2	0.0949
3	ExHSU	1	0.1029	4	HSU	2	[redacted]
7	ExHSU	1	0.0782	6	HSU	2	0.1178
10	ExHSU	1	0.0749	13	HSU	2	0.0838
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.1071	30	HSU	2	0.1092
33	ExHSU	1	0.1031	31	HSU	2	0.0981
34	ExHSU	1	[redacted]	32	HSU	2	0.1038
41	ExHSU	1	0.0908	36	HSU	2	0.0720
45	ExHSU	1	0.0848	38	HSU	2	0.0963
46	ExHSU	1	0.0972	43	HSU	2	0.0748
49	ExHSU	1	[redacted]	47	HSU	2	0.1013
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	0.0866	5	C	4	0.1218
12	Ex	3	0.1144	9	C	4	0.0759
14	Ex	3	0.1119	15	C	4	0.1098
17	Ex	3	0.0978	16	C	4	0.1007
20	Ex	3	0.1363	18	C	4	0.0844
24	Ex	3	0.1082	19	C	4	0.0901
25	Ex	3	0.1084	21	C	4	0.1230
29	Ex	3	0.1101	26	C	4	0.1101
37	Ex	3	0.1010	27	C	4	0.1299
42	Ex	3	0.1160	35	C	4	0.0919
44	Ex	3	0.0972	39	C	4	0.1175
48	Ex	3	0.1175	40	C	4	0.1117

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table B.2. Soleus dry weight.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	0.0571	1	HSU	2	0.0599
3	ExHSU	1	0.0614	4	HSU	2	[redacted]
7	ExHSU	1	0.0491	6	HSU	2	0.0903
10	ExHSU	1	0.0431	13	HSU	2	0.0463
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.0863	30	HSU	2	0.0777
33	ExHSU	1	0.0692	31	HSU	2	0.1010
34	ExHSU	1	[redacted]	32	HSU	2	0.0880
41	ExHSU	1	0.0724	36	HSU	2	0.0562
45	ExHSU	1	0.0781	38	HSU	2	0.1142
46	ExHSU	1	0.0616	43	HSU	2	0.0654
49	ExHSU	1	[redacted]	47	HSU	2	0.0916
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	0.0595	5	C	4	0.0899
12	Ex	3	0.0906	9	C	4	0.0550
14	Ex	3	0.0725	15	C	4	0.0737
17	Ex	3	0.0626	16	C	4	0.0716
20	Ex	3	0.1209	18	C	4	0.0583
24	Ex	3	0.0801	19	C	4	0.0557
25	Ex	3	0.0905	21	C	4	0.1029
29	Ex	3	0.0938	26	C	4	0.0887
37	Ex	3	0.0806	27	C	4	0.1053
42	Ex	3	0.0979	35	C	4	0.0831
44	Ex	3	0.0674	39	C	4	0.0862
48	Ex	3	0.1035	40	C	4	0.0956

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table B.3. Soleus wet-weight to body-weight ratio.

Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)	Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)
2	ExHSU	1	0.4206	1	HSU	2	0.4413
3	ExHSU	1	0.4635	4	HSU	2	[redacted]
7	ExHSU	1	0.4600	6	HSU	2	0.5479
10	ExHSU	1	0.4005	13	HSU	2	0.4190
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.5301	30	HSU	2	0.5571
33	ExHSU	1	0.5078	31	HSU	2	0.4762
34	ExHSU	1	[redacted]	32	HSU	2	0.5242
41	ExHSU	1	0.4127	36	HSU	2	0.3529
45	ExHSU	1	0.4240	38	HSU	2	0.4542
46	ExHSU	1	0.4909	43	HSU	2	0.3561
49	ExHSU	1	[redacted]	47	HSU	2	0.4668
Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)	Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)
11	Ex	3	0.4373	5	C	4	0.5389
12	Ex	3	0.4349	9	C	4	0.4264
14	Ex	3	0.4761	15	C	4	0.4374
17	Ex	3	0.4817	16	C	4	0.4683
20	Ex	3	0.5609	18	C	4	0.4097
24	Ex	3	0.5032	19	C	4	0.4527
25	Ex	3	0.4479	21	C	4	0.5168
29	Ex	3	0.4606	26	C	4	0.4439
37	Ex	3	0.4549	27	C	4	0.5367
42	Ex	3	0.4754	35	C	4	0.3894
44	Ex	3	0.4320	39	C	4	0.4936
48	Ex	3	0.4855	40	C	4	0.4753

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.

Table B.4. Extensor digitorum longus wet weight.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	0.1025	1	HSU	2	0.0933
3	ExHSU	1	0.0935	4	HSU	2	[redacted]
7	ExHSU	1	0.0683	6	HSU	2	0.0793
10	ExHSU	1	0.0780	13	HSU	2	0.0807
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.0941	30	HSU	2	0.0977
33	ExHSU	1	0.1098	31	HSU	2	0.0910
34	ExHSU	1	[redacted]	32	HSU	2	0.0857
41	ExHSU	1	0.0869	36	HSU	2	0.0800
45	ExHSU	1	0.0914	38	HSU	2	0.0938
46	ExHSU	1	0.1069	43	HSU	2	0.0821
49	ExHSU	1	[redacted]	47	HSU	2	0.0940
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	0.0753	5	C	4	0.0907
12	Ex	3	0.1156	9	C	4	0.0702
14	Ex	3	0.0990	15	C	4	0.1064
17	Ex	3	0.0821	16	C	4	0.0810
20	Ex	3	0.1028	18	C	4	0.0779
24	Ex	3	0.0958	19	C	4	0.0697
25	Ex	3	0.1080	21	C	4	0.0817
29	Ex	3	0.1168	26	C	4	0.1136
37	Ex	3	0.1006	27	C	4	0.1255
42	Ex	3	0.1184	35	C	4	0.0931
44	Ex	3	0.0936	39	C	4	0.0931
48	Ex	3	0.0953	40	C	4	0.0901

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table B.5. Extensor digitorum longus dry weights.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	0.0654	1	HSU	2	0.0641
3	ExHSU	1	0.0737	4	HSU	2	[redacted]
7	ExHSU	1	0.0446	6	HSU	2	0.0428
10	ExHSU	1	0.0470	13	HSU	2	0.0496
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.0718	30	HSU	2	0.0790
33	ExHSU	1	0.0767	31	HSU	2	0.0485
34	ExHSU	1	[redacted]	32	HSU	2	0.0284
41	ExHSU	1	0.0542	36	HSU	2	0.0569
45	ExHSU	1	0.0600	38	HSU	2	0.0732
46	ExHSU	1	0.0783	43	HSU	2	0.0587
49	ExHSU	1	[redacted]	47	HSU	2	0.0761
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	0.0585	5	C	4	0.0607
12	Ex	3	0.0832	9	C	4	0.0463
14	Ex	3	0.0777	15	C	4	0.0839
17	Ex	3	0.0594	16	C	4	0.0625
20	Ex	3	0.0670	18	C	4	0.0354
24	Ex	3	0.0624	19	C	4	0.0322
25	Ex	3	0.0914	21	C	4	0.0586
29	Ex	3	0.0854	26	C	4	0.0808
37	Ex	3	0.0645	27	C	4	0.0801
42	Ex	3	0.0768	35	C	4	0.0801
44	Ex	3	0.0707	39	C	4	0.0676
48	Ex	3	0.0827	40	C	4	0.0757

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table B.6. Extensor digitorum longus wet-weight to body-weight ratio.

Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)	Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)
2	ExHSU	1	0.4812	1	HSU	2	0.4339
3	ExHSU	1	0.4211	4	HSU	2	[redacted]
7	ExHSU	1	0.4017	6	HSU	2	0.3688
10	ExHSU	1	0.4171	13	HSU	2	0.4035
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.4658	30	HSU	2	0.4984
33	ExHSU	1	0.5408	31	HSU	2	0.4417
34	ExHSU	1	[redacted]	32	HSU	2	0.4328
41	ExHSU	1	0.3950	36	HSU	2	0.3921
45	ExHSU	1	0.4570	38	HSU	2	0.4424
46	ExHSU	1	0.5398	43	HSU	2	0.3909
49	ExHSU	1	[redacted]	47	HSU	2	0.4331
Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)	Rat #	Name	Group	Relative Weight ($g \cdot kg^{-1}$ BW)
11	Ex	3	0.3803	5	C	4	0.4013
12	Ex	3	0.4395	9	C	4	0.3943
14	Ex	3	0.4212	15	C	4	0.4239
17	Ex	3	0.4044	16	C	4	0.3767
20	Ex	3	0.4230	18	C	4	0.3781
24	Ex	3	0.4455	19	C	4	0.3502
25	Ex	3	0.4462	21	C	4	0.3432
29	Ex	3	0.4887	26	C	4	0.4580
37	Ex	3	0.4531	27	C	4	0.5185
42	Ex	3	0.4852	35	C	4	0.3944
44	Ex	3	0.4160	39	C	4	0.3911
48	Ex	3	0.3938	40	C	4	0.3834

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

APPENDIX C. ENZYME ACTIVITY

Table C.1. Soleus glucose-6-phosphate dehydrogenase activity.

Rat #	Name	Group	G-6-PDH Activity (μmole·g^{-1}·min^{-1})	Rat #	Name	Group	G-6-PDH Activity (μmole·g^{-1}·min^{-1})
2	ExHSU	1	3.5787	1	HSU	2	3.9138
3	ExHSU	1	3.0763	4	HSU	2	[redacted]
7	ExHSU	1	3.6977	6	HSU	2	2.2789
10	ExHSU	1	1.8956	13	HSU	2	4.4855
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	4.7568	30	HSU	2	4.8292
33	ExHSU	1	3.8133	31	HSU	2	2.1975
34	ExHSU	1	[redacted]	32	HSU	2	7.3975
41	ExHSU	1	3.6189	36	HSU	2	4.0876
45	ExHSU	1	2.3995	38	HSU	2	3.9339
46	ExHSU	1	4.4614	43	HSU	2	3.1617
49	ExHSU	1	[redacted]	47	HSU	2	6.0229
Rat #	Name	Group	G-6-PDH Activity (μmole·g^{-1}·min^{-1})	Rat #	Name	Group	G-6-PDH Activity (μmole·g^{-1}·min^{-1})
11	Ex	3	2.6362	5	C	4	2.4101
12	Ex	3	1.6178	9	C	4	2.9843
14	Ex	3	1.6494	15	C	4	2.4206
17	Ex	3	1.5685	16	C	4	1.8006
20	Ex	3	2.5161	18	C	4	2.3392
24	Ex	3	3.4968	19	C	4	0.6210
25	Ex	3	2.3352	21	C	4	3.7138
29	Ex	3	3.7490	26	C	4	2.3649
37	Ex	3	1.4017	27	C	4	3.3883
42	Ex	3	2.9085	35	C	4	2.3563
44	Ex	3	4.5684	39	C	4	3.1778
48	Ex	3	4.2620	40	C	4	2.1071

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table C.2. Extensor digitorum longus glucose-6-phosphate dehydrogenase activity.

Rat #	Name	Group	G-6-PDH Activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)	Rat #	Name	Group	G-6-PDH Activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)
2	ExHSU	1	1.8850	1	HSU	2	0.6224
3	ExHSU	1	2.4477	4	HSU	2	[redacted]
7	ExHSU	1	1.7765	6	HSU	2	1.3112
10	ExHSU	1	3.1440	13	HSU	2	0.6888
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	1.6117	30	HSU	2	1.9272
33	ExHSU	1	1.3585	31	HSU	2	0.8425
34	ExHSU	1	[redacted]	32	HSU	2	2.1452
41	ExHSU	1	1.7182	36	HSU	2	1.1133
45	ExHSU	1	1.2600	38	HSU	2	0.5777
46	ExHSU	1	1.4348	43	HSU	2	2.7692
49	ExHSU	1	[redacted]	47	HSU	2	2.4819
Rat #	Name	Group	G-6-PDH Activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)	Rat #	Name	Group	G-6-PDH Activity ($\mu mole \cdot g^{-1} \cdot min^{-1}$)
11	Ex	3	1.5253	5	C	4	0.0000
12	Ex	3	2.3377	9	C	4	1.9905
14	Ex	3	1.6529	15	C	4	2.2106
17	Ex	3	2.7577	16	C	4	1.3555
20	Ex	3	1.9734	18	C	4	1.3715
24	Ex	3	1.6278	19	C	4	0.4381
25	Ex	3	1.8111	21	C	4	1.7735
29	Ex	3	0.6752	26	C	4	0.9947
37	Ex	3	1.5675	27	C	4	1.5836
42	Ex	3	0.9867	35	C	4	1.4896
44	Ex	3	0.4571	39	C	4	0.0000
48	Ex	3	1.5253	40	C	4	0.8757

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

APPENDIX D. MUSCLE HISTOLOGY

Table D.1. Soleus Fiber Area.

Rat #	Name	Group	Fiber Area (μm^2)	Rat #	Name	Group	Fiber Area (μm^2)
2	ExHSU	1	1513.41	1	HSU	2	1396.03
3	ExHSU	1	1881.98	4	HSU	2	[redacted]
7	ExHSU	1	1419.23	6	HSU	2	1984.08
10	ExHSU	1	1580.86	13	HSU	2	1349.22
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	1519.08	30	HSU	2	3460.05
33	ExHSU	1	2250.84	31	HSU	2	2274.27
34	ExHSU	1	[redacted]	32	HSU	2	1964.49
41	ExHSU	1	2384.57	36	HSU	2	1970.84
45	ExHSU	1	4077.47	38	HSU	2	2043.31
46	ExHSU	1	2071.70	43	HSU	2	1966.40
49	ExHSU	1	[redacted]	47	HSU	2	2526.97
Rat #	Name	Group	Fiber Area (μm^2)	Rat #	Name	Group	Fiber Area (μm^2)
11	Ex	3	1927.12	5	C	4	1984.08
12	Ex	3	3688.12	9	C	4	2012.69
14	Ex	3	2896.34	15	C	4	[redacted]
17	Ex	3	3342.39	16	C	4	2456.80
20	Ex	3	3512.88	18	C	4	4080.57
24	Ex	3	3250.82	19	C	4	4408.19
25	Ex	3	2211.93	21	C	4	2669.62
29	Ex	3	3360.49	26	C	4	2499.90
37	Ex	3	3178.20	27	C	4	2556.64
42	Ex	3	2815.96	35	C	4	2816.03
44	Ex	3	3274.65	39	C	4	2810.33
48	Ex	3	3274.65	40	C	4	2809.97

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.

Note: Blackened numbers represent data excluded from analysis.

Note: Rat 15 excluded from analysis due to an error in histological preparation for the cross section.

Table D.2. Soleus fiber area to body-weight ratio.

Rat #	Name	Group	Ratio	Rat #	Name	Group	Ratio
2	ExHSU	1	.7105	1	HSU	2	.6493
3	ExHSU	1	.8477	4	HSU	2	[redacted]
7	ExHSU	1	.8348	6	HSU	2	.9228
10	ExHSU	1	.8454	13	HSU	2	.6746
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	.7520	30	HSU	2	1.7653
33	ExHSU	1	1.1088	31	HSU	2	1.1040
34	ExHSU	1	1.5223	32	HSU	2	.9922
41	ExHSU	1	1.0839	36	HSU	2	.9661
45	ExHSU	1	2.0387	38	HSU	2	.9638
46	ExHSU	1	1.0463	43	HSU	2	.9364
49	ExHSU	1	[redacted]	47	HSU	2	1.1645
Rat #	Name	Group	Ratio	Rat #	Name	Group	Ratio
11	Ex	3	.9733	5	C	4	.8779
12	Ex	3	1.4023	9	C	4	1.1307
14	Ex	3	1.2325	15	C	4	[redacted]
17	Ex	3	1.6465	16	C	4	1.1427
20	Ex	3	1.4456	18	C	4	1.9809
24	Ex	3	1.5120	19	C	4	2.2152
25	Ex	3	.9140	21	C	4	1.1217
29	Ex	3	1.4061	26	C	4	1.0080
37	Ex	3	1.4316	27	C	4	1.0565
42	Ex	3	1.1541	35	C	4	1.1932
44	Ex	3	1.4554	39	C	4	1.1808
48	Ex	3	1.2764	40	C	4	1.1957

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.
Note: Rat 15 excluded from analysis due to an error in histological preparation for the cross section.

Table D.3. Soleus percent myofibrillar damage.

Rat #	Name	Group	Fiber Damage (%)	Rat #	Name	Group	Fiber Damage (%)
2	ExHSU	1	2.183	1	HSU	2	13.095
3	ExHSU	1	1.488	4	HSU	2	[redacted]
7	ExHSU	1	4.455	6	HSU	2	0.259
10	ExHSU	1	0.473	13	HSU	2	4.427
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	1.002	30	HSU	2	5.276
33	ExHSU	1	4.657	31	HSU	2	2.834
34	ExHSU	1	[redacted]	32	HSU	2	8.982
41	ExHSU	1	1.407	36	HSU	2	2.205
45	ExHSU	1	0.640	38	HSU	2	0.738
46	ExHSU	1	2.444	43	HSU	2	9.933
49	ExHSU	1	[redacted]	47	HSU	2	2.587
Rat #	Name	Group	Fiber Damage (%)	Rat #	Name	Group	Fiber Damage (%)
11	Ex	3	0.000	5	C	4	0.000
12	Ex	3	0.012	9	C	4	3.047
14	Ex	3	0.000	15	C	4	0.019
17	Ex	3	0.039	16	C	4	0.039
20	Ex	3	0.004	18	C	4	0.241
24	Ex	3	0.017	19	C	4	0.185
25	Ex	3	0.000	21	C	4	0.070
29	Ex	3	0.223	26	C	4	0.029
37	Ex	3	0.119	27	C	4	0.002
42	Ex	3	0.007	35	C	4	0.000
44	Ex	3	0.000	39	C	4	0.045
48	Ex	3	0.014	40	C	4	0.090

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table D.4. Soleus longitudinal total fiber area.

Rat #	Name	Group	Fiber Area (mm^2)	Rat #	Name	Group	Fiber Area (mm^2)
2	ExHSU	1	161909.0	1	HSU	2	138595.6
3	ExHSU	1	183331.4	4	HSU	2	[redacted]
7	ExHSU	1	170044.1	6	HSU	2	163416.8
10	ExHSU	1	188152.6	13	HSU	2	172694.7
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	184532.8	30	HSU	2	176090.9
33	ExHSU	1	176473.6	31	HSU	2	183387.8
34	ExHSU	1	[redacted]	32	HSU	2	182544.5
41	ExHSU	1	170962.2	36	HSU	2	170106.9
45	ExHSU	1	174731.5	38	HSU	2	189202.1
46	ExHSU	1	187987.2	43	HSU	2	183561.0
49	ExHSU	1	[redacted]	47	HSU	2	187770.6
Rat #	Name	Group	Fiber Area (mm^2)	Rat #	Name	Group	Fiber Area (mm^2)
11	Ex	3	194081.1	5	C	4	164942.3
12	Ex	3	200491.7	9	C	4	154239.4
14	Ex	3	174097.2	15	C	4	170715.1
17	Ex	3	190103.2	16	C	4	183133.7
20	Ex	3	200149.0	18	C	4	188619.3
24	Ex	3	205100.0	19	C	4	186709.6
25	Ex	3	181273.7	21	C	4	173811.7
29	Ex	3	195101.3	26	C	4	230826.3
37	Ex	3	154074.9	27	C	4	199949.4
42	Ex	3	196132.1	35	C	4	209048.1
44	Ex	3	163133.4	39	C	4	196349.2
48	Ex	3	175418.7	40	C	4	174953.8

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table D.5. Soleus percentage of interstitial area.

Rat #	Name	Group	Interstitial Area (%)	Rat #	Name	Group	Interstitial Area (%)
2	ExHSU	1	4.17	1	HSU	2	22.83
3	ExHSU	1	8.11	4	HSU	2	[redacted]
7	ExHSU	1	14.43	6	HSU	2	16.02
10	ExHSU	1	14.63	13	HSU	2	33.09
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	1.73	30	HSU	2	3.21
33	ExHSU	1	11.82	31	HSU	2	12.42
34	ExHSU	1	[redacted]	32	HSU	2	14.78
41	ExHSU	1	10.60	36	HSU	2	6.47
45	ExHSU	1	4.87	38	HSU	2	8.61
46	ExHSU	1	5.05	43	HSU	2	9.84
49	ExHSU	1	[redacted]	47	HSU	2	3.50
Rat #	Name	Group	Interstitial Area (%)	Rat #	Name	Group	Interstitial Area (%)
11	Ex	3	8.85	5	C	4	9.68
12	Ex	3	2.29	9	C	4	16.68
14	Ex	3	6.87	15	C	4	[redacted]
17	Ex	3	10.92	16	C	4	19.17
20	Ex	3	4.12	18	C	4	15.68
24	Ex	3	13.45	19	C	4	17.55
25	Ex	3	17.65	21	C	4	9.42
29	Ex	3	6.65	26	C	4	6.43
37	Ex	3	2.70	27	C	4	6.77
42	Ex	3	2.13	35	C	4	4.24
44	Ex	3	3.38	39	C	4	4.59
48	Ex	3	6.69	40	C	4	8.89

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.

Note: Blackened numbers represent data excluded from analysis.

APPENDIX E. ADRENAL GLAND WEIGHTS

Table E.1. Adrenal Gland Weights.

Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
2	ExHSU	1	0.0895	1	HSU	2	0.0998
3	ExHSU	1	0.0721	4	HSU	2	[redacted]
7	ExHSU	1	0.0659	6	HSU	2	0.0710
10	ExHSU	1	0.0751	13	HSU	2	0.0780
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	0.0801	30	HSU	2	0.0710
33	ExHSU	1	0.0649	31	HSU	2	0.0610
34	ExHSU	1	[redacted]	32	HSU	2	0.0572
41	ExHSU	1	0.0829	36	HSU	2	0.0559
45	ExHSU	1	0.0684	38	HSU	2	0.0742
46	ExHSU	1	0.0680	43	HSU	2	0.0682
49	ExHSU	1	[redacted]	47	HSU	2	0.0816
Rat #	Name	Group	Weight (g)	Rat #	Name	Group	Weight (g)
11	Ex	3	0.0578	5	C	4	0.0754
12	Ex	3	0.0861	9	C	4	0.0783
14	Ex	3	0.0625	15	C	4	0.0971
17	Ex	3	0.0649	16	C	4	0.0695
20	Ex	3	0.0735	18	C	4	0.0430
24	Ex	3	0.0589	19	C	4	0.0586
25	Ex	3	0.0839	21	C	4	0.0723
29	Ex	3	0.0738	26	C	4	0.0765
37	Ex	3	0.0650	27	C	4	0.0683
42	Ex	3	0.0601	35	C	4	0.0611
44	Ex	3	0.0603	39	C	4	0.0613
48	Ex	3	0.0701	40	C	4	0.0710

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

Table E.2. Adrenal weight to body-weight ratios.

Rat #	Name	Group	Ratio	Rat #	Name	Group	Ratio
2	ExHSU	1	.3248	1	HSU	2	.4202
3	ExHSU	1	.4535	4	HSU	2	[redacted]
7	ExHSU	1	.4399	6	HSU	2	.3877
10	ExHSU	1	.2919	13	HSU	2	.2660
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	.3088	30	HSU	2	.2961
33	ExHSU	1	.2970	31	HSU	2	.2889
34	ExHSU	1	[redacted]	32	HSU	2	.3197
41	ExHSU	1	.2463	36	HSU	2	.2928
45	ExHSU	1	.3434	38	HSU	2	.2576
46	ExHSU	1	.3760	43	HSU	2	.2680
49	ExHSU	1	[redacted]	47	HSU	2	.2897
Rat #	Name	Group	Ratio	Rat #	Name	Group	Ratio
11	Ex	3	.3274	5	C	4	.3302
12	Ex	3	.3900	9	C	4	.4016
14	Ex	3	.3869	15	C	4	.3233
17	Ex	3	.2087	16	C	4	.3197
20	Ex	3	.3038	18	C	4	.2944
24	Ex	3	.3380	19	C	4	.3025
25	Ex	3	.3085	21	C	4	.3421
29	Ex	3	.3622	26	C	4	.2822
37	Ex	3	.3500	27	C	4	.3965
42	Ex	3	.3248	35	C	4	.2589
44	Ex	3	.3420	39	C	4	.3021
48	Ex	3	.3180	40	C	4	.3768

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

APPENDIX F. TIBIA LENGTHS

Table F.1. Tibia lengths.

Rat #	Name	Group	Lengths (mm)	Rat #	Name	Group	Lengths (mm)
2	ExHSU	1	38.30	1	HSU	2	38.30
3	ExHSU	1	35.90	4	HSU	2	[redacted]
7	ExHSU	1	35.20	6	HSU	2	34.00
10	ExHSU	1	36.10	13	HSU	2	36.10
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	40.00	30	HSU	2	40.00
33	ExHSU	1	39.90	31	HSU	2	37.20
34	ExHSU	1	[redacted]	32	HSU	2	40.70
41	ExHSU	1	39.20	36	HSU	2	39.00
45	ExHSU	1	39.20	38	HSU	2	39.30
46	ExHSU	1	38.30	43	HSU	2	39.70
49	ExHSU	1	[redacted]	47	HSU	2	38.30
Rat #	Name	Group	Lengths (mm)	Rat #	Name	Group	Lengths (mm)
11	Ex	3	32.50	5	C	4	38.10
12	Ex	3	35.70	9	C	4	36.60
14	Ex	3	38.20	15	C	4	36.30
17	Ex	3	33.80	16	C	4	38.70
20	Ex	3	39.40	18	C	4	[redacted]
24	Ex	3	38.30	19	C	4	[redacted]
25	Ex	3	39.00	21	C	4	38.20
29	Ex	3	39.80	26	C	4	40.00
37	Ex	3	38.90	27	C	4	38.80
42	Ex	3	39.40	35	C	4	40.10
44	Ex	3	39.80	39	C	4	39.70
48	Ex	3	39.80	40	C	4	39.10

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

APPENDIX G. TIBIA BONE MINERAL CONTENT

Table G.1. Tibia bone mineral content.

Rat #	Name	Group		Rat #	Name	Group	
2	ExHSU	1	.2500	1	HSU	2	.2360
3	ExHSU	1	.2036	4	HSU	2	[redacted]
7	ExHSU	1	.1573	6	HSU	2	.1564
10	ExHSU	1	.1721	13	HSU	2	.1847
22	ExHSU	1	[redacted]	23	HSU	2	[redacted]
28	ExHSU	1	.2591	30	HSU	2	.2773
33	ExHSU	1	.2557	31	HSU	2	.2244
34	ExHSU	1	[redacted]	32	HSU	2	.2742
41	ExHSU	1	.2504	36	HSU	2	.2247
45	ExHSU	1	.2246	38	HSU	2	.2507
46	ExHSU	1	.2300	43	HSU	2	.2421
49	ExHSU	1	[redacted]	47	HSU	2	.2479
Rat #	Name	Group		Rat #	Name	Group	
11	Ex	3	.1497	5	C	4	.2253
12	Ex	3	.2095	9	C	4	.1795
14	Ex	3	.2298	15	C	4	.2090
17	Ex	3	.1692	16	C	4	.2118
20	Ex	3	.2545	18	C	4	[redacted]
24	Ex	3	.2380	19	C	4	[redacted]
25	Ex	3	.2633	21	C	4	.2841
29	Ex	3	.2291	26	C	4	.2814
37	Ex	3	.2354	27	C	4	.2775
42	Ex	3	.2851	35	C	4	.2557
44	Ex	3	.2485	39	C	4	.2654
48	Ex	3	.2794	40	C	4	.2584

ExHSU, exercise + hindlimb suspension unloading; HSU, hindlimb suspension unloading; Ex, exercise only; C, control.
Note: Blackened numbers represent data excluded from analysis.

APPENDIX H. RELIABILITY

Twenty-five computer files were made from slides consisting of longitudinal sections of rat soleus muscles. The Scion image program was used to determine the reliability (within and between testers) of assessing fiber area of normal vs. damaged skeletal muscle. Tissue was considered damaged if the striated banding pattern was disrupted similarly to what is observed following unaccustomed eccentric exercise [Fridén, 1980 #18] or following a period of unloading [Riley, 1990 #50; Riley, 1992 #52]. Fiber area was considered normal unless it displayed this type of banding pattern (i.e. the banding pattern was wide and/or overstretched). The testers were provided detailed instructions and examples of the type of fiber area considered damaged vs. normal and each examiner analyzed the same computer files on three consecutive days. The order in which the files were analyzed on each test day was randomly chosen prior to analysis. Normal fiber area (μm^2) and damaged fiber area (μm^2) was determined for each computer file separately and mixed model ANOVAs were used to determine intraclass correlation coefficients within and between testers. The results are presented in Table H.1.

Table H.1. Intraclass correlation coefficients within and between Testers.

Tester	Trials (#)	Undamaged Area	Damaged Area
1	3	r = .99	r = .99
2	3	r = .98	r = .95
3	3	r = .98	r = .99
Between	3	r = .98	r = .95

APPENDIX I. DISSERTATION IMAGES

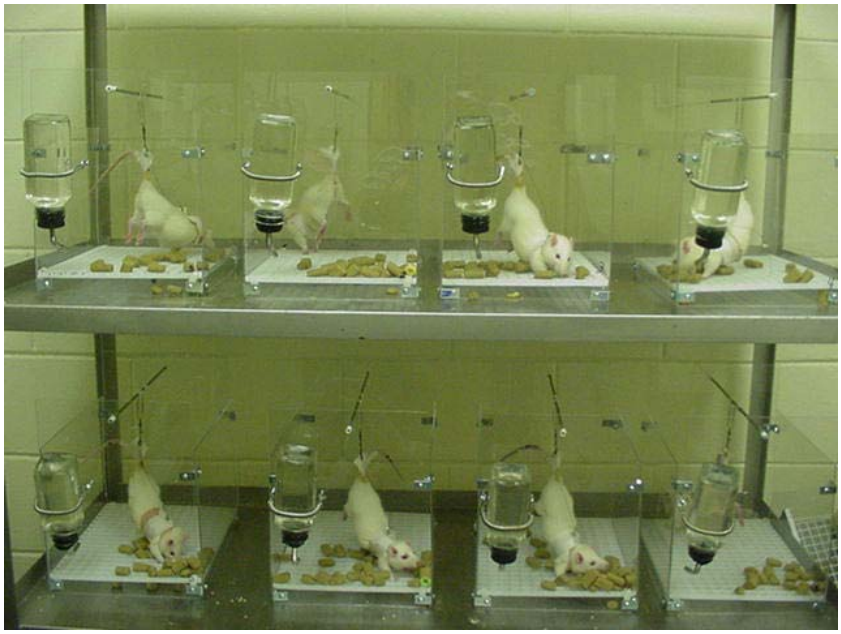


Figure I.1. Hindlimb suspended animals during one stage of data collection.

Figure I.2. Suspension cage and hindlimb suspension unloading model.

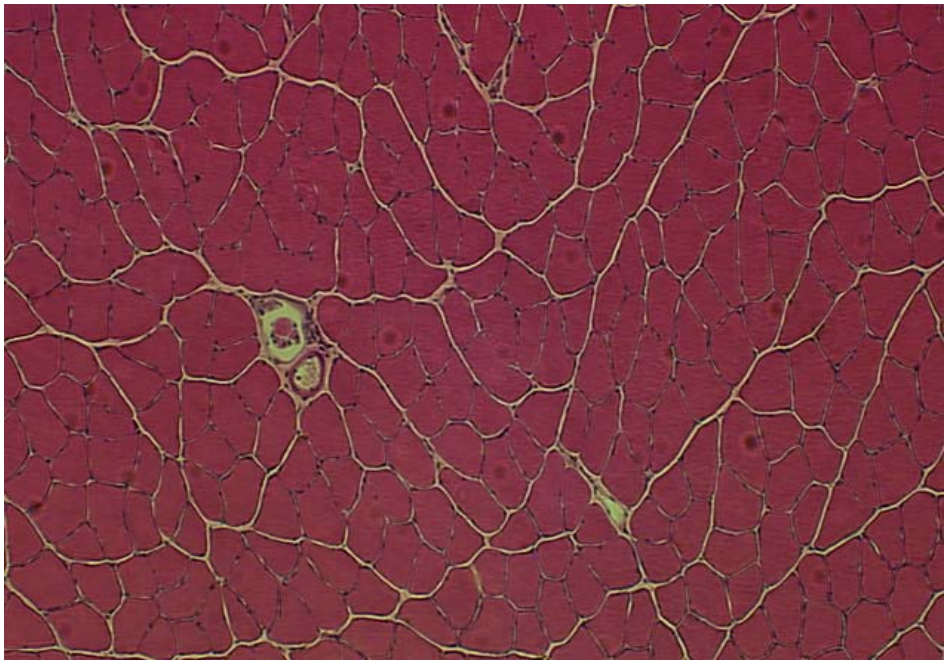


Figure I.3. Phosphotungstic acid-hematoxylin-stained cross section of SOL muscle in control rat. x 40.

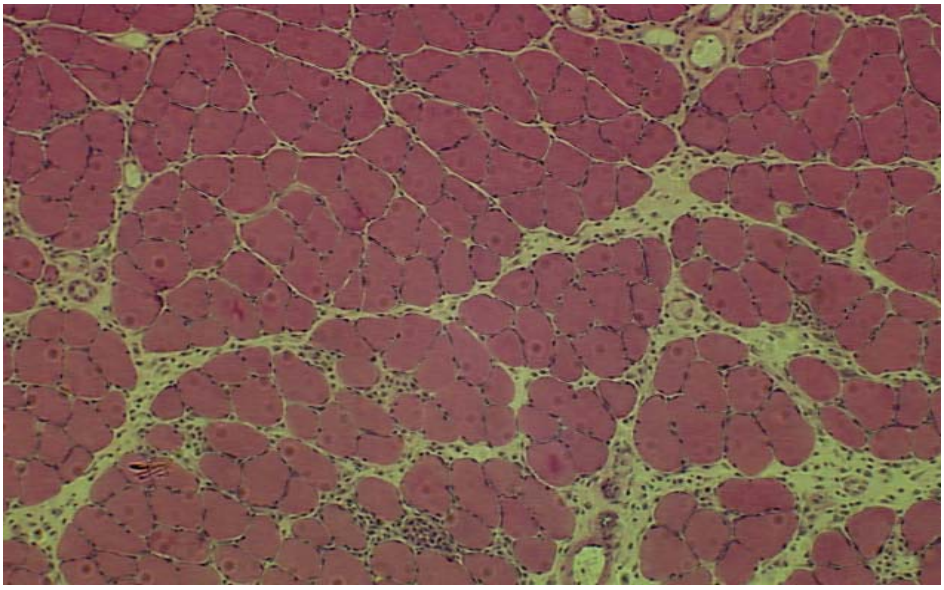


Figure I.4. Phosphotungstic acid-hematoxylin-stained cross section of SOL muscle following 7 d HSU + 16-19 h reloading. Note the larger area occupied by interstitial space. x40.

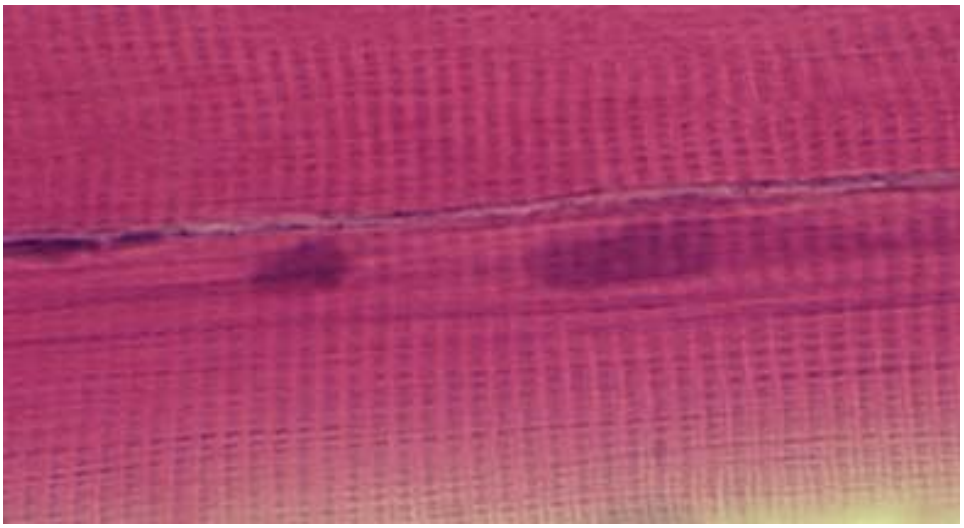


Figure I.5. Phosphotungstic acid-hematoxylin-stained longitudinal section of SOL muscle in control rat. Normal cross striations are easily observed. x100.

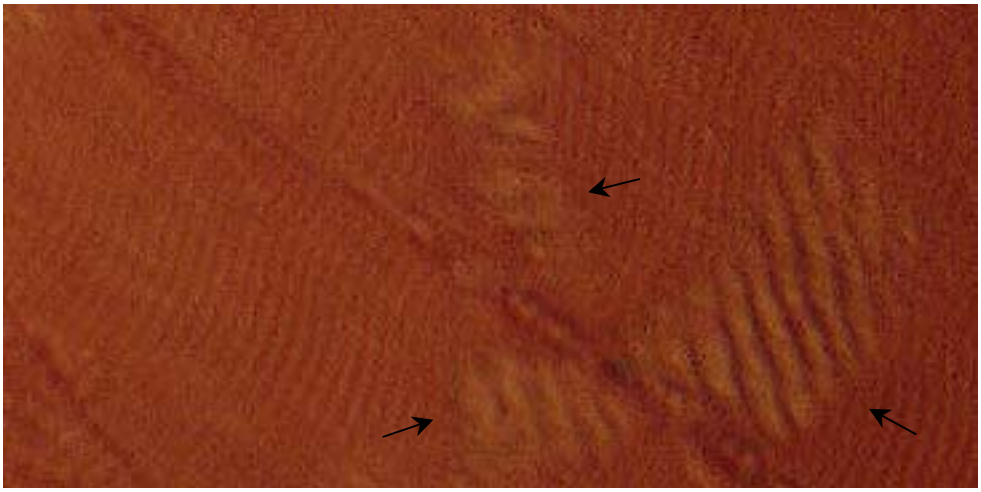


Figure I.6. Phosphotungstic acid-hematoxylin-stained longitudinal section of SOL muscle following 7 d of HSU + 16-19 h of reloading. Normal cross striations are easily observed. Arrows point to pale foci of widened cross striations, which represents eccentric contraction-like lesions. x100.

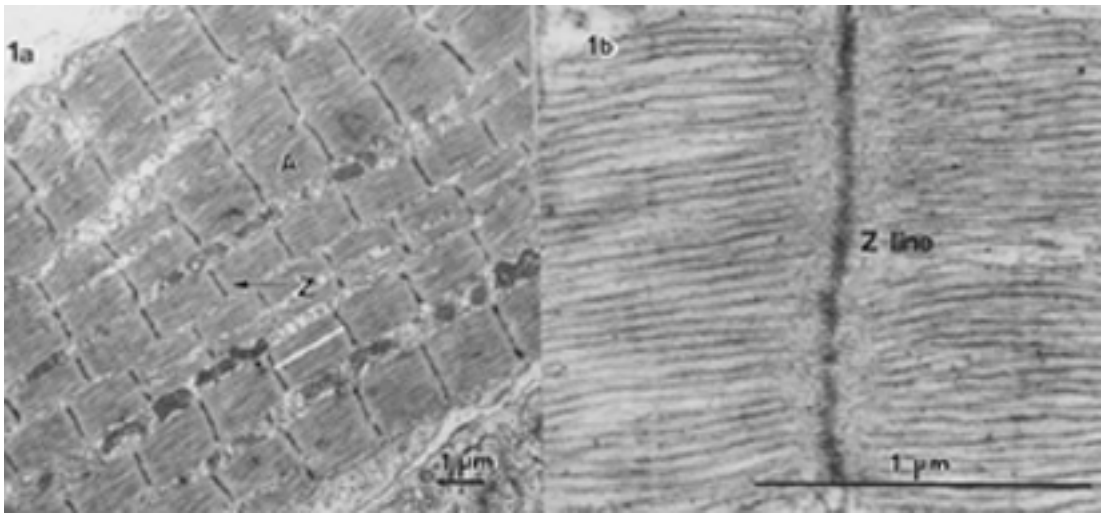


Figure I.7. Electron micrograph of a ultra-thin longitudinal section of a control SOL muscle.
1a. Normal sarcomeres with regularly spaced Z-lines (Z) are easily observed and arranged in a parallel fashion. The letter A represents the A-band. x5600. 1b. A higher magnification of a normal SOL control muscle. A normal Z-line is easily observed. x55,000. 1 μm = 1 micrometer.

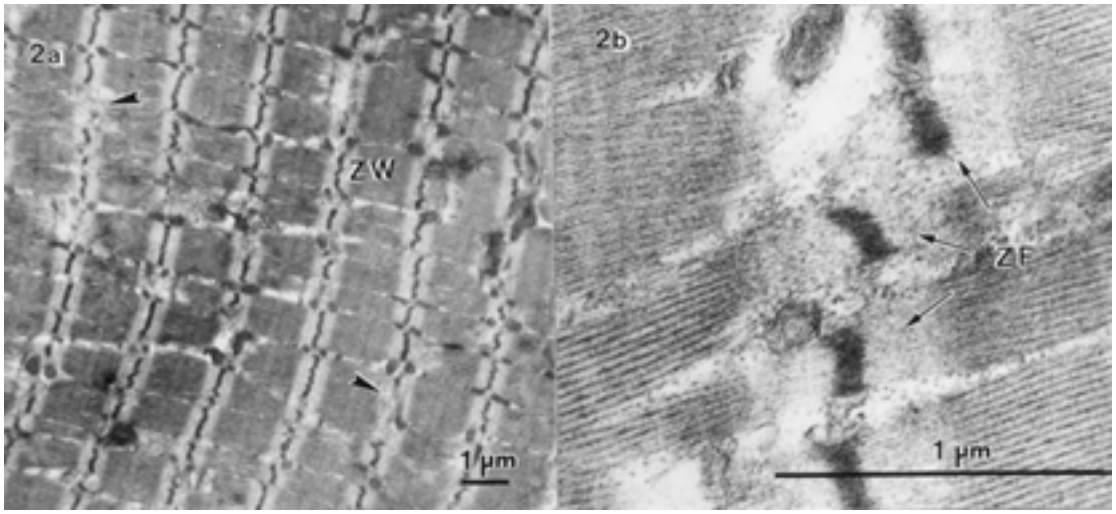


Figure I.8. Electron micrograph of a ultra-thin longitudinal section of a SOL muscle following 7 d HSU and 16-19 h of reloading.
2a. Note the wider and wavier Z-lines (ZW) and less distinct appearance between the contractile filaments in the A-band. Pale, diminished Z-lines (arrows) are also evident throughout the region. x5600. 2b. Areas of Z-line “fracture” (ZF) are evident at a higher magnification. X55,000. 1 μm = 1 micrometer.

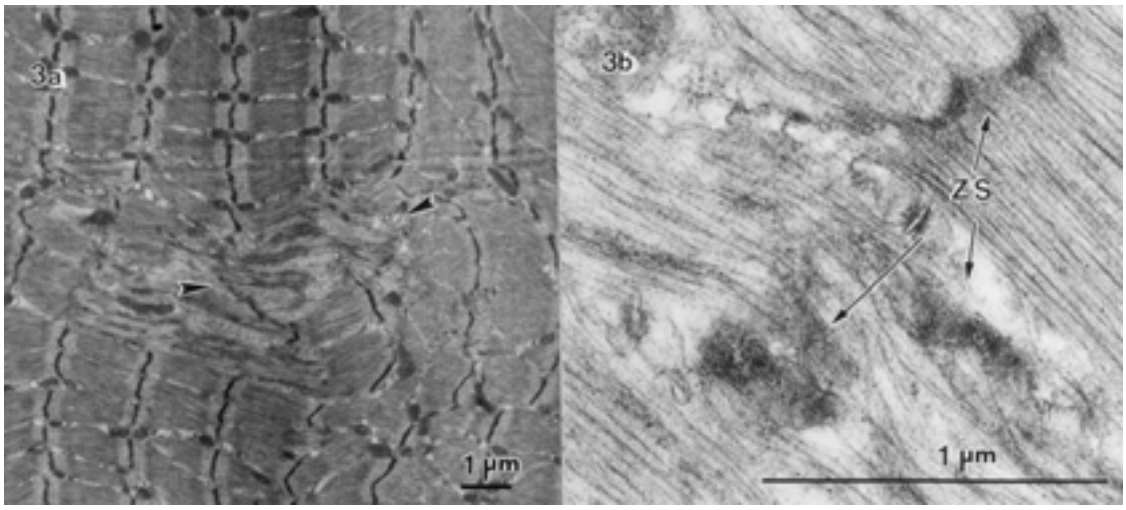


Figure I.9. Electron micrograph of an ultra-thin longitudinal section of a SOL muscle following 7 d HSU and 16-19 h of reloading.
3a. Severe sarcomere disruption is evident. Individual sarcomeres appear stretched and pulled out of register with sarcomeres above and below the damaged region (arrows). The normal cross-striated pattern that can be viewed in Figure I.7 (1a) is not evident in the damaged region of this micrograph. x5600.
3b. Higher magnification further illustrates Z-line "streaming" (ZS) as Z-line-like dense material extends longitudinally into the sarcomere, interfering with contractile filaments in the A-band. x55,000. 1 μm = 1 micrometer.

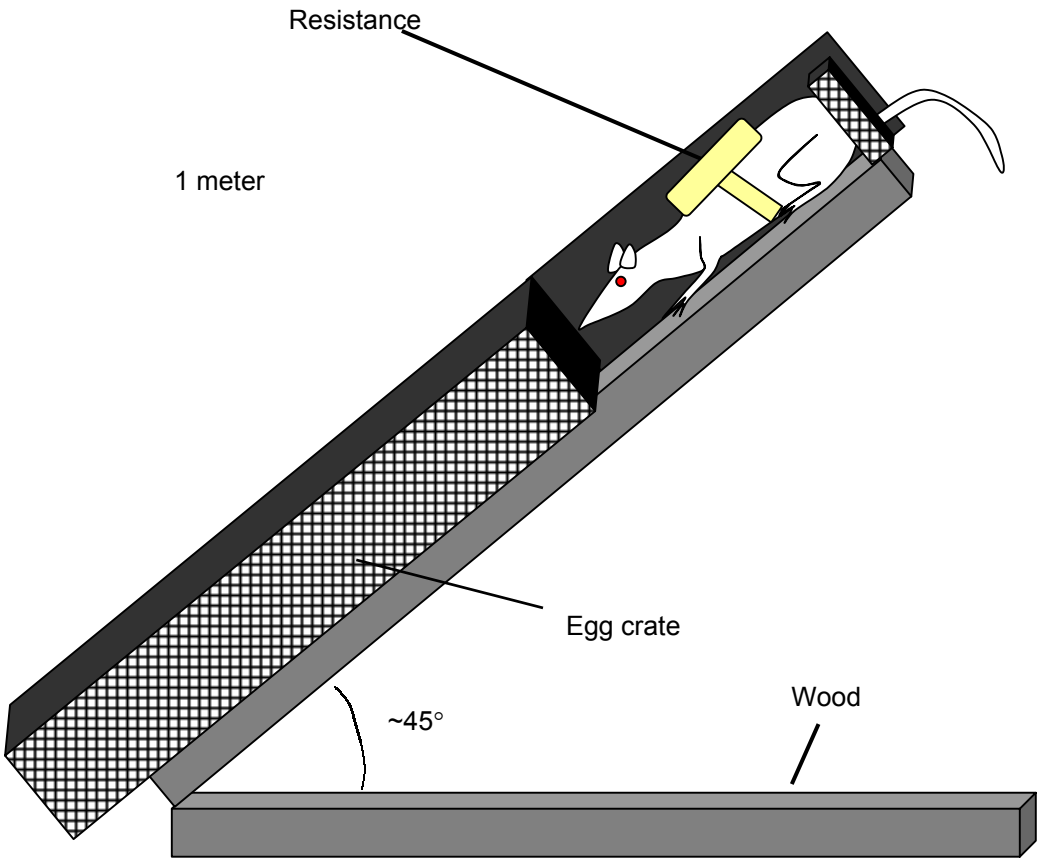


Figure I.10. Exercise apparatus.

APPENDIX J. METHODOLOGY FOR ASSESSING MUSCLE DAMAGE

Criteria for Assessing Skeletal Muscle Damage from Electron Micrographs

Control and experimental rat soleus muscles were prepared for electron microscopy. One hundred and ten sarcomeres were randomly chosen from 11 electron micrographs (x 5600) to determine damaged (D) vs. Undamaged (U) sarcomeres and to assess the magnitude of damage. Sarcomeres were considered damaged if Z-lines were abnormal [wavy, fragmented, missing or streaming] and if abnormalities were observed within the sarcomere [tear(s), infiltration, disruption of striated pattern, and/or overstretched in size]. The magnitude of damage was quantified by assessing the overall appearance of the sarcomere according to the given criteria and reports of ultrastructural skeletal muscle damage.

Protocol

1. The sarcomeres to be assessed were randomly chosen beforehand and written down on the test sheet under the heading Sarcomere (e.g. A6, B1, etc.).

2. A grid was lain overtop of the micrograph. This grid was labeled alphabetically across the top and numbered along the left side.

3. The sarcomere assessed was located by simply following the vertical and horizontal lines until they intersected one another.

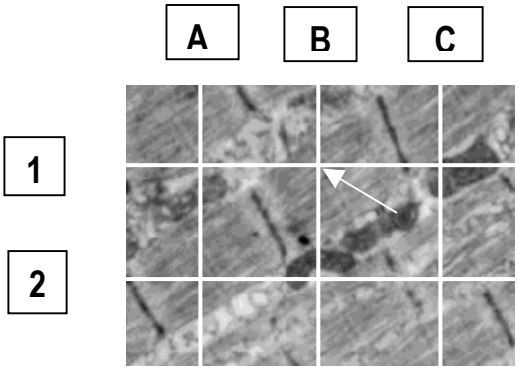
4. At this point, the sarcomere was assessed according to the instructions given.

5. For example, sarcomere (B1) was assessed accordingly:

    a. Follow the vertical line B until it intersects with the horizontal line #1.

b. The sarcomere to be assessed lies directly underneath this point (denoted by arrow). This is sarcomere B1.

c. Only this sarcomere was assessed.



Assessing Damage

1. One and only one sarcomere was assessed per point.

2. First, the Z-lines were assessed by looking at both the left and right lines and scoring them separately.

3. According to the definitions, the examiner chose the definition that best fit what was observed (definitions given below).

4. For example, if the left Z-line appeared wavy, an L was placed in the cell below the Wavy category and the right Z-line appeared to be streaming, an R was placed in the cell underneath that category. (See example below).

5. If the definitions did not represent the Z-lines or if the Z-lines appeared normal, the cell(s) were left blank.

6. Again, only one category per Z-line was chosen (if a category is chosen).

Z-Line Appearance			
Wavy	Fragmented	Missing	Streaming
L			R

7. The overall appearance of the sarcomere (except for the Z-lines) was assessed accordingly:

a. An X was placed in the cell for EACH abnormality observed.

b. Unlike assessment of the Z-Lines, all categories that applied to the appearance of the sarcomere were checked.

c. For example, if the sarcomere was infiltrated by an organelle and overstretched above normal size, an X was placed in the cells below those categories (See example below).

d. If an abnormality was observed that was not represented in the definitions given, an X was placed in the cell underneath Other (see example below).

Sarcomere Appearance (excluding Z-lines)				*Other*
Torn	Infiltrated	Disrupted	Overstretched	
	X		X	X

Definitions

Z-Lines

a. Wavy – The Z-line is dark and continuous (not broken) but has a wavy appearance along the length of the sarcomere.

b. Fragmented – The Z-line is dark, but appears broken or disrupted along its length (include Z-lines that do not traverse the entire sarcomere).

c. Missing (Opaque) – the entire Z-line is no longer visible along side the A-band or the density of the Z-line is nearly faded away.

d. Streaming – the Z-line has lost the appearance of a line as dark Z-line-like material is extended into surrounding A-bands.

Sarcomere Appearance

a. Torn – There is a tear or several tears within the sarcomere.

b. Infiltrated – the sarcomere is infiltrated by other organelles (e.g. mitochondria, lipid droplets, etc.).

c. Disrupted – the normal actin and myosin striations are disrupted or disorganized [exclude infiltration by organelles and tear(s)].

d. Overstretched – the sarcomere appears to be overstretched or larger than surrounding “normal” sarcomeres.

e. Other – mark this if tester notices any other abnormality that is not represented by the definitions above. Make sure to briefly describe the abnormality and why you feel that it doesn’t belong in the above definitions.

Sarcomere Scoring

Z-lines:		Sarcomere Appearance:	
Wavy	1	Torn	5
Fragmented	2	Infiltrated	4
Missing (Opaque)	3	Disrupted	3
Streaming	4	Overstretched	2
		Other	1

VITA

Rhonda Dianne Prisby was born on October 10, 1969, in Warren, Ohio. She attended the public school system in Leavittsburg, Ohio, and graduated in 1988 from LaBrae High School. During her high school years, she was a varsity member of the basketball, softball and track and field teams. She continued to play varsity basketball and softball for Hiram College in Hiram, Ohio, where she enrolled in the fall of 1988. She graduated in 1992 with a Bachelor of Arts in Studio Art.

Rhonda's interest in athletics and exercise prompted her to enroll at Kent State University in 1994, where she completed the degree of a master of Arts in exercise physiology. During this time, she completed a thesis in the area of thermoregulation and body composition in young women and graduated in the summer of 1997.

Rhonda enrolled in the Department of Kinesiology at Louisiana Sate University in Baton Rouge, Louisiana in the fall of 1997. She has pursed a doctorate in kinesiology with a major emphasis in exercise physiology and a minor emphasis in nutrition. In additional to departmental research, she conducted research in the Human Ecology Department. Her major research interest includes the effects of inactivity on skeletal muscle atrophy and damage, and has prompted much of her work to date. Upon graduation in August of 2002 with the degree of doctor of philosophy, she plans to begin work on a post-doctorate position in the

Department of Health and Kinesiology at Texas Agricultural and Mechanical University in College Station, Texas.